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TOXIC AND PRIORITY ORGANICS IN MUNICIPAL SLUDGE LAND TREATMENT SYSTEMS

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16. ABSTRACT

The goal of the research reported herein was to begin a methodical investigation of organic priority pollutants applied to plant-soil systems at rates characteristic of municipal sludge land treatment. A single chemical was applied at rates of 0.1, 10, and 100-fold of the expected value received during an annual application of municipal sludge. The ^{14}C-chemicals investigated were in the following groups: polynuclear aromatics, phthalic acid esters, and substituted aromatic compounds. None of the organic priority pollutants studied was entirely excluded from all plant species at the rates of soil application utilized. The ratio of vegetation fresh weight concentration of a chemical to the concentration loaded initially onto the soil (bioaccumulation) was most typically less than 0.01 and always less than 1.0. Of the crops studied (fescue, corn, soybeans, wheat), no vegetation type was routinely the species evidencing the highest uptake of the organic chemicals used. Plant uptake appears to be largely governed by the losses over time from the soil and the water solubility of a given chemical. The presence of sludge did not appear to significantly alter the crop uptake or soil loss of these compounds. If corroborated, this information would allow a large expansion of design information based on available literature information.

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimonies to the deterioration of our natural environment. The complexity of that environment and the interplay of its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution; it involves defining the problem, measuring its impact, and searching for solutions. The Water Engineering Research Laboratory develops new and improved technology and systems to prevent, treat, and manage wastewater and solid and hazardous waste pollutiont discharges from municipal and community sources, to preserve and treat public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research and provides a most vital communications link between the researcher and the user community.

This report is the first formal Water Engineering Research Laboratory research effort aimed at the behavior of organic priority pollutants under municipal sludge land treatment conditions. The information resulting from this research is valuable in assessing the efficacy of the important practice of municipal sludge land treatment.

Francis T. Mayo, Director
Water Engineering Research Laboratory

ABSTRACT

The goal of the research reported herein was to begin a methodical investigation of organic priority pollutants applied to plant soil systems at rates characteristic of municipal sludge land treatment. A single chemical was applied at rates of 0, 1, 10, and 100-fold of the expected value received during an annual application of municipal sludge. The ^{14}C-chemicals investigated were in the following groups, polynuclear aromatics, phthalic acid esters, and substituted aromatic compounds. None of the organic priority pollutants studied was entirely excluded from all plant species at the rates of soil application utilized. The ratio of vegetation concentration of a chemical to the concentration loaded initially onto the soil (bioaccumulation) was most typically less than 0.01 and always less than 1.0. Of the crops studied (fescue, corn, soybeans, wheat), no vegetation type was routinely the species evidencing the highest uptake of the organic chemicals used. Plant uptake appears to be largely governed by the losses over time from the soil and the water solubility of a given chemical.

The presence of sludge did not appear to significantly alter the crop uptake or soil loss of these compounds. If corroborated, this information would allow a large expansion of design information based on available literature information.

CONTENTS

Foreword iii
Abstract iv
Figures ix
Tables x
Acknowledgments xiv

1. INTRODUCTION 1
    References 2

2. CONCLUSIONS 3

3. RECOMMENDATIONS 6

4. EXPERIMENTAL PROCEDURES 7
    Organic priority pollutant selection and loading rates 7
    Experiment location and facilities 11
    Materials and protocols 12
        chemicals 12
        experiment container 12
        soil 15
        container loading 17
        plant establishment 17
        preliminary studies 17
        routine planting and germination protocols 21
        maintenance of experiment 23
        harvesting 24
        experiment termination 24
    Sampling and verification used in experiments 25
        soil loading verification 25
        soil and crop tests for extraction efficiency 25
        soil sampling at end of experiment 25
        crop sampling for routine analysis 26
        crop evaluation of root:shoot ratio 26
        crop studies of chemical translocation 28
        crop evaluation for consistency of sampling and analysis ... 28
        crop studies to verify presence of parent compounds 28
    Summary of sample nomenclature 29
    References 30

5. CROP PLANT GROWTH, UPTAKE, AND SOIL RETENTION OF PHTHALIC ACID ... 31
    Introduction 31
    Materials and methods 32
        chemicals 32
        crop plants 32
        chemical analysis 32
        extraction efficiencies 33
        plant uptake and soil concentration calculations 33

thin layer chromatography (TLC) 34
statistical analysis 34
Results 35
plant germination and growth 35
plant uptake 35
soil retention 41
Conclusions 41
References 43

6. CROP UPTAKE AND SOIL RETENTION OF PHTHALIC ACID ESTERS: DI-n-BUTYL PHTHLATE (DnBP), DI-n-OCTYL PHTHALATE (DnOP), AND DIETHYL HEXYL PHTHALATE (DEHP) 46
Materials and methods 46
chemical 46
crop plants 48
chemical analysis 48
extraction efficiencies 49
chemical concentration calculations 49
statistical analysis 49
Results and discussion 50
plant growth 50
plant uptake 50
soil retention 50
Conclusions 57
References 59

7. CROP UPTAKE AND SOIL RETENTION OF PHTHALIC ACID ESTERS: DI-n-BUTYL PHTHALATE AND DI-ETHYL PHTHALATE 60
Materials and methods 60
chemical 60
crop plants 60
chemical analysis 60
extraction efficiencies 62
chemical concentration calculations 62
statistical analysis 64
Results 64
plant growth 64
plant uptake 64
soil retention 71
Conclusions 71

8. CROP PLANT GROWTH AND UPTAKE OF POLYNUCLEAR AROMATICS: ANTHRACENE, BENZ(A)ANTHRACENE, NAPHTHALENE, AND PHENANTHRENE 74
Introduction 74
Materials and methods 74
chemicals 74
crop plants 75
chemical analysis 75
extraction efficiencies 76
benz(a)anthracene disappearance studies 76
^{14}C extraction efficiencies 77
thin layer chromatography 77

        gas chromatographic analysis 77
        chemical concentration calculations 78
        statistical analysis 78
    Results 79
        plant growth 79
        plant uptake 79
        soil retention 86
        ^{14}C balance 90
    Conclusions 90
    References 92

9. PLANT GROWTH, UPTAKE, AND SOIL RETENTION OF SUBSTITUTED AROMATIC; 14 CHLOROBENZENE AND THE EFFECT OF SLUDGE 93
    Materials and methods 93
        chemicals 93
        crop plants 93
        chemical analysis 93
        chemical concentration calculations 94
        statistical analysis 95
    Results 95
        plant growth 95
        plant uptake 95
        soil retention 99
    Conclusions 99

10. PLANT GROWTH, UPTAKE, AND SOIL RETENTION OF SUBSTITUTED AROMATICS: PARADICHLOROBENZENE AND THE EFFECT OF SLUDGE 104
    Materials and methods 104
        chemicals 104
        crop plants 104
        chemical analysis 104
        chemical concentration calculations 105
        statistical analysis 106
    Results 106
        plant growth 106
        plant uptake 106
        soil retention 108
    Conclusions 108
    References 114

11. PLANT GROWTH, UPTAKE, AND SOIL RETENTION OF DI-n-BUTYL PHTHALATE ACID ESTER AND THE EFFECT OF MUNICIPAL SLUDGE 116
    Materials and methods 116
        chemicals 116
        crop plants 116
        chemical analysis 116
        chemical concentration calculations 116
        statistical analysis 117
    Results 117
        soil 118
        plant growth 118
        plant uptake 123
    Conclusions 123

12. SUMMARY OF EXPERIMENTS 132

FIGURES

Number		Page
1	Number of organic (nonpesticide) priority pollutants in various municipal sludges	9
2	Experimental containers for crop and soil studies	14
3	Experimental pot configuration of plants	20
4	Soil retention of polynuclear aromatics - extractable	89
5	Percent of original ^{14}C-DnBP extractable from soil	119
6	Percent of original ^{14}C-DnBP oxidizable from soil	121
7	Uptake (ppb) of extractable, oxidizable and total ^{14}C-DnBP in soybean plants grown in sludge amended pots at 28 days	126
8	Uptake (ppb) of total (oxidation and extraction) ^{14}C-DnBP in plants grown in sludge amended pots at 73 days	129
9	Generalized relationship of organic chemical uptake by plants	135

TABLES

Number Page

1 Organic (Nonpesticide) Priority Pollutant Analysis of Municipal Sludge 8

2 General Grouping of Organic Priority Pollutants (Nonpesticides) Found in Top Ten Concentration Levels for Ten Municipal Sludges 10

3 Experimental Schedule and Greenhouse Use 13

4 Norfolk Sandy Loam Properties 16

5 Effect of Solvent on Seed Germination in Norfolk Soil ... 18

6 Germination of Seeds Used in Three Year Study 22

7 Root:Shoot Ratios at Experiment Termination 27

8 Percent Germination of Plants Grown in Greenhouse Pots Treated with Phthalic Acid 36

9 Number and Standard Deviation of Plants at Harvest in Greenhouse Pots Treated with Phthalic Acid 37

10 Dry Weight (gm), Height (cm) and Standard Deviation of Plants Grown in Greenhouse Pots Treated with Phthalic Acid 38

11 Uptake and Standard Deviation of Extractable Phthalic Acid in Greenhouse-Grown Plants (ppb-dry weight) 39

12 Regressions of Plant Uptake and Soil Retention of Phthalic Acid 40

13 Amount and Standard Deviation of Extractable Phthalic Acid Remaining in Soil After Final Harvest (74 days) 42

14 Amounts of Phthalic Acid Esters Applied to Pots in Greenhouse Studies 47

15 Height (cm) and Standard Deviations at Harvest of Plants Grown in Greenhouse Pots Treated with Phthalic Acid Esters 51

16 Dry Weight (gm) and Standard Deviations of Plants Grown in Greenhouse Pots Treated with Phthalic Acid Esters 52

17 Dry Weight (gm) and Standard Deviations of Plants Grown in Greenhouse Pots Treated with Phthalic Acid Esters 53

18 Uptake of Phthalic Acid Esters as Measured ^{14}C in Greenhouse-Grown Plants (ppb) 54

19 Uptake of Phthalic Acid Esters as Measured ^{14}C in Greenhouse-Grown Plants (ppb) 55

20 Top Soil Retention of Phthalic Acid Esters as Measured ^{14}C (ppb) 56

21 Total DPM Recovered at End of Experiment and Percent of Total DPM Recovered in Aboveground Plant Tissue 58

22 Amounts of Phthalate Acid Esters Applied to Pots in Greenhouse Studies 61

23 ^{14}C-Phthalate Acid Esters - Percent Extraction Efficiencies 64

24 Height (cm) and Standard Deviations of Plants Grown in Greenhouse Pots Treated with Phthalic Acid Esters 65

25 Height (cm) and Standard Deviations of Plants Grown in Greenhouse Pots Treated with Phthalic Acid Esters 66

26 Dry Weight (gm) and Standard Deviations of Plants Grown in Greenhouse Pots Treated with Phthalic Acid Esters 67

27 Dry Weight (gm) and Standard Deviations of Plants Grown in Greenhouse Pots Treated with Phthalic Acid Esters 68

28 Uptake of Phthalic Acid Esters as Measured ^{14}C in Greenhouse-Grown Plants (ppb) 69

29 Uptake of Phthalic Acid Esters as Measured ^{14}C in Greenhouse-Grown Plants (ppb) 70

30 Soil Retention of Phthalic Acid Esters as Measured ^{14}C (ppb) 72

31 Total ^{14}C-DPM Recovered at the End of the Experiment and Percent of Total ^{14}C-DPM Recovered in Aboveground Plant Tissue 73

32 Percent Soil Leaching of DPM 73

33 ^{14}C-Polynuclear Aromatic Chemical Extraction Efficiencies 76

34 Height (cm) and Standard Deviations of Plants Grown in Greenhouse Pots Treated with Polynuclear Aromatics 80

35 Dry Weight (gm) and Standard Deviations of Plants Grown in Greenhouse Pots Treated with Polynuclear Aromatics 81

36 Percent ^{14}C Extraction Efficiency From Plants and Soil in Greenhouse Grown Pots 82

37 Uptake of Polynuclear Aromatics in Greenhouse Grown Plants--parts per billion dry weight 83

38 Regression Equations of Plant Uptake of Polynuclear Aromatics 84

39 Soil Retention of Polynuclear Aromatics After Final Harvest--parts per billion dry weight 87

40 Percent of Original ^{14}C PNA's Recovered in Bottom Soil 88

41 Total ^{14}C Recovered at End of the Experiment and Percent of Total ^{14}C Recovered in Aboveground Plant Tissue 91

42 Height (cm) of Plants at Harvest Grown in Greenhouse Pots with Chlorobenzene and Sewage Sludge 96

43 Dry Weight (gm) per Pot of Plants at Harvest Grown in Greenhouse Pots with Chlorobenzene and Sewage Sludge 97

44 Statistical Significance and Sources of Significance (F value) of Height (cm) of Plants in Chlorobenzene Amended Pots 100

45 Plant Uptake of Extractable ^{14}C-Chlorobenzene in Plants Grown in Greenhouse Pots (ppb) 101

46 Soil Nutrients, Heavy Metals and pH of Control Pots 102

47 Oxidizable ^{14}C-Chlorobenzene from Soil in Greenhouse Pots - ppb Remaining and Percent of Original Applied Radiation Left 103

48 Height (cm) and Standard Deviation of Plants at Harvest Grown in Greenhouse Pots with p-Dichlorobenzene 107

49 Dry Weight (gm) and Standard Deviation of Plants Grown inGreenhouse Pots with p-Dichlorobenzene 109

50 Uptake and Standard Deviations (ppb) of Extractable ^{14}C p-Dichlorobenzene in Plants Grown in Greenhouse Pots 111

51 Soil Nutrients, Heavy Metals and pH of Control Pots 112

52 Soil Concentrations of ^{14}C and p-Dichlorobenzene 113

53 Mass Balance of Extractable ^{14}C from p-Dichlorobenzene Applied in Greenhouse Pots - Percent of Original Applied ^{14}C 115

54 Summary of Statistical Significance of ^{14}C DnBP (extracted and total) in Soil 120

55 Soil ^{14}C Extraction Efficiency 122

56 Number of Soybean Plants, Height (mm), Dry Weight (gm) and Standard Deviations of Plants Harvested at 23 Days 124

57 Number of Soybean Plants, Height (mm), Dry Weight (gm) and Standard Deviations of Plants Harvested at 73 Days 125

58 Summary of Statistical Significance of ^{14}C-DnBP (extracted and total) in Plants 127

59 Plant ^{14}C Extraction Efficiency from 28 Day Plants 128

60 Plant ^{14}C Extraction Efficiency from 73 Day Plants 130

61 Summary of Selected Data from Experiments in this Research Project 133

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SECTION I

INTRODUCTION

The historical perspective regarding the specific organic constituents in municipal effluents or sludges is somewhat varied. Using the Proceedings of the Joint Conference on Recycling Municipal Sludges and Effluents on Land (U.S. E.P.A. 1972) as a benchmark of the municipal land treatment field, one would conclude that there had been minimal attention on these waste constituents. However, one must review the situation in 1972 for an interpretation of this lack of attention.

In 1972 there was no list of organic compounds that had been proposed to be of environmental concern. Thus, only certain organics were identified as possible concerns, namely the Aroclor mixtures referred to broadly as polychlorinated biphenyls (PCB). These were mentioned in the chapters on municipal effluent and sludge characterization and in the chapter on monitoring (Blakeslee 1972). Actual quantification of Aroclor concentrations were given for a number of municipal effluents and sludges in Michigan. Citations in this benchmark Proceedings represented the level of attention focused on specific organics in regard to municipal land application.

A different perspective of the concern and level of understanding for organic compounds is obtained by evaluating the broader field of the behavior of organic compounds in the terrestrial system (even at the 1972 period). This broader field encompasses research interests in pesticides and residues, other agricultural chemicals, soil sterilization, response of chemical spills, organic fertilizers, and fundamental investigations of soil-plant behavior (Weber 1978). With this broader perspective, one concludes that there is a very large data base and a substantial delineation of mechanisms operative for specific organic compounds in soil-plant systems (Weber and Sheets 1978). This information began accumulating in early 1900 and the article by Buddin (1914) represents (even at that early date) a sound understanding of germicidal effects of organic chemicals on soils (some of which are now considered priority pollutants). Thus, the research into the behavior of toxic and nontoxic organic compounds when applied to land has been continued as a significant research area today. This contrasts with the low level of attention given this area in relation to land treatment, circa 1972.

The magnitude and diversity of the organic compounds that have been evaluated in the context of a terrestrial system was estimated recently and put in catalogue form, called TERRETOX (Overcash and Sims 1981). From an article-by-article search of 240 appropriate journals for the period 1976-1981, over 4,000 organic compounds were found (many nonpesticides). These citations contained one or more evaluations of the following classes of terrestrial behavior of specific organics: volatilization, photolysis, decomposition, adsorption, plant uptake, phytotoxicity. Thus there exists a substantial (and, to many, a surprisingly large) data base from which to begin evaluation of specific organic compounds as these might impact land application of municipal effluents and sludges.

In 1976 the definition of organic compounds of environmental concern changed from an amorphous collection containing a few highly visible chemicals to a specific list of organics - the Consent Decree Priority Pollutants. Prior to this date, attention on organics in municipal wastes was restricted by the lack of knowledge about what compounds were present or might be of concern. Thus, attention or research priority was limited in scope. With the priority pollutant list, whether complete or not, a program was undertaken to measure these specific organics in municipal wastes, effluents and sludges (Feiler 1980). These results are discussed in a later section. For the organic chemical class, the increased awareness of municipal effluent and sludge concentrations of organic constituents has stimulated recognition of the larger body of research information pertinent to understanding this facet of land application. Thus, the merging of information from the terrestrial behavior of organic compounds to land application of municipal sludge and effluents is beginning. Given the extent of the former, it appears that there will be significant transferable information to allow estimation of behavior, relative assimilation capacities, and critical levels for specific organics. However, specific research on the organic priority pollutants in the presence of the municipal sludge or effluent will also be necessary as the basic understanding of the individual chemicals is obtained (experimentally or via the literature).

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SECTION 2

CONCLUSIONS

During the course of this research project, preferential emphasis has been given to the crop uptake, with decomposition of organic priority pollutants in soil-plant systems being of secondary importance. These pathways are a part of a complex behavior undergone by organic chemicals in the terrestrial environment. In overview, the fate of organic priority pollutants is a process of loss through a variety of chemical and biological routes with the rate of loss depending on the particular compound.

Conclusions from the experiments reported herein are drawn as much as possible from the integration of data derived from the following chemicals: phthalate acid esters [bis (2 ethylhexyl), di-n-octyl, di-n-butyl, and di ethyl]; polynuclear aromatics (naphthalene, anthracene, phenanthrene, and benz(a)anthracene); and chlorinated aromatics (chlorobenzene and p-dichlorobenzene). The conclusions are:

1. Measurement of radiolabel (which can be parent or metabolites) was used as an indication of the behavior of the parent ^{14}C compound. Some verification of ^{14}C as parent compound was performed.

2. After a full growth cycle, a certain fraction of ^{14}C and possibly parent compound remained unextractable from the plants.

3. All organic compounds studied were taken up by plants, but the bioaccumulation factors appear significantly less than one. This is viewed as a major safety factor since with the losses of an organic chemical (e.g., decomposition), successive crops have reduced uptake approaching the lowest level of detection.

4. For the three organic chemical groups studied, the bioaccumulation factor (vegetation fresh weight concentration: concentration loaded on soil) was always less than 1.0, most often was less than 0.1, and in the majority of cases was less than 0.01.

5. No single crop (among corn, fescue, soybean, and wheat) was routinely the species with the highest uptake.

6. Competing phenomena of loss from the soil and solubility (bioavailability) appeared to control the levels of an organic chemical taken up by vegetation. These phenomena are complex.

7. In experiments with three organic compounds, the presence of municipal sludge as a large organic addition did not appear to significantly alter the crop uptake or decomposition of the organic chemicals. Should these results prove even qualitatively correct, large amounts of available information on

organic compounds in terrestrial systems could be used in estimating he effects of specific organics on Lund disposal of sludges.

8. Organic compound studies in municipal sludge land treatment systems will remain complex, expensive, and time-consuming investigations for the near future.

SECTION 3

RECOMMENDATIONS

The future direction of research on organic chemicals in municipal sludge land treatment systems will benefit from continued emphasis on measurements. Several important needs appear as areas of research:

1. Investigations should be initiated to expand the number of groups of organic chemicals in which representative compounds are evaluated in relation to the behavior in soil-plant systems;
2. A continued perspective must be maintained among the various constituents of municipal sludge (e.g., pathogens metals, organics, anions) when evaluating or designing municipal sludge land treatment systems.
3. With the limited information available as a part of this report, and with data more broadly available, preliminary consideration of the risk sequence probable with municipal sludge and crops grown on such systems should be undertaken.

SECTION 4

EXPERIMENTAL PROCEDURES

ORGANIC PRIORITY POLLUTANT SELECTION AND LOADING RATES

At the beginning of this project, the data on priority pollutants were in the early phase of availability. Preliminary results were available (June 1980) from the Effluent Guidelines Division of the U.S. Environmental Protection Agency (report EPA-440/1-79-300). Concentrations of priority pollutants in sludge were reported for eleven cities on a wet basis.

Examination of individual municipalities indicated that not all of the 89 organic (nonpesticide) pollutants were present. For the eleven sludges, the percent of the 89 organic priority pollutants found averaged 30% with a range of 20-40%. Thus, the selection of study compounds was narrowed. The next evaluation concerned frequency of occurence and concentrations. The organics were ranked from most concentrated to least concentrated, eliminating compounds below the detection limit. An example of this ranking is Table 1. For the eleven cities, the number of organic pollutants averaged 20 species and ranged from 13 to 33, (Figure 1). Two sludges were taken at different locations within the treatment plant at several of these cities. The clear bar graph represents the chemicals common to both sample locations, while the two cross-hatched portions correspond to chemicals not common at the two locations within the treatment plant. Expressing the percentage of common pollutants to the total number of pollutants found, one can assess the effect of a treatment plant on diversity of pollutants. The total number of pollutants is the sum of the two bar heights minus the height of the clear section. For the first municipality with multiple samples, the total number of organic priority pollutants is 30. For the five municipalities with multiple sludge analyses, the percentage of common parameters was 58% with a range of 40-72%. Thus, a single sludge was not a good measure of other sludge sources although there was a majority of organic pollutants that were common. Substantial numbers of compounds are thus appearing and disappearing at various locations of the sludge processing. These differences tend to be for organic compounds which are less frequently present when compared across all data collected.

From an experimental perspective, it was important to use groups of similar chemicals to simplify analytical tasks and resources expenditures. Thus, an evaluation of the frequency of occurence was made to select the groups of chemicals for investigation. These results are given in Table 2. Five groups were thus identified in which a particular chemical was included if the compound was found with reasonable frequency from the eleven city study. Three groups were selected for study based on time and project resources: the phthalate acid esters, the polynuclear aromatics, and the substituted benzene compounds.

TABLE 1. ORGANIC (NONPESTICIDE) PRIORITY POLLUTANT ANALYSIS OF MUNICIPAL SLUDGE

Primary Sludge

Pollutant	Concentration, (μg/l)
bis(2-ethylhexyl) phthalate	2230
anthracene	1570
phenanthrene	1570
pyrene	757
3,4-benzofluoranthene	675
1,2-benzanthracene	479
chrysene	479
fluorene	313
tetrachloroethylene	293
toluene	284
trichloroethylene	284
ethylbenzene	276
methylene chloride	222
naphthalene	195
benzene	171
acenaphthene	169
phenol	94
pentachlorophenol	93
dichlorobromomethane	57
1,1,1-trichloroethane	24
1,2-trans-dichloroethylene	23
chlorodibromomethene	17
carbon tetrachloride	11
1,1-dichloroethane	11
1,1-dichloroethylene	9
butylbenzylphthalate	1

Secondary Sludge

Pollutant	Concentration, (μg/l)
methylene chloride	249
pentachlorophenol	112
phenol	68
dichlorobromomethane	56
bis(2-ethylhexyl) phthalate	42
chlorodibromomethane	29
1,2:5,6-dibenzanthracene	10
benzene	10
indeno(1,2,3-C,D) pyrene	8
tetrachloroethylene	7
carbon tetrachloride	6
phenanthrene	4
anthracene	4
naphthalene	4
ethylbenzene	4
acrylonitrile	3
toluene	2
trichloroethylene	1

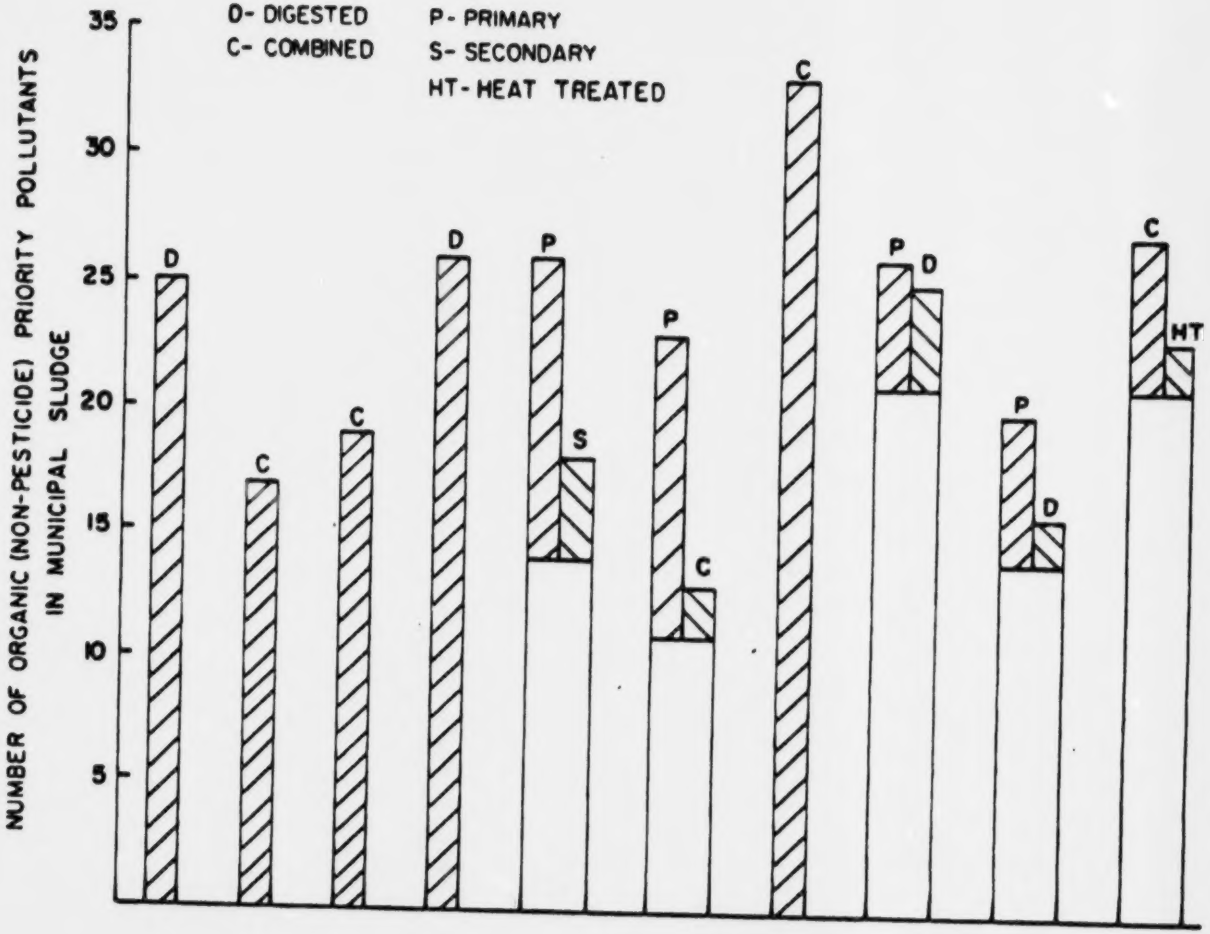

Figure 1. Number of organic (nonpesticide) priority pollutants in various municipal sludges.

TABLE 2. GENERAL GROUPING OF ORGANIC PRIORITY POLLUTANTS (NONPESTICIDES) FOUND IN TOP TEN CONCENTRATION LEVELS FOR TEN MUNICIPAL SLUDGES

Phthalate Esters

bis (2-ethylhexyl) phthalate
butyl benzyl phthalate
di-n-butyl phthalate
di-n-octyl phthalate

Polynuclear Aromatics

naphthalene
fluorene
anthracene
phenanthrene
pyrene
3,4 benzofluoranthene
fluoranthene
1,2 benzanthracene
chrysene

Substituted Benzene Compounds

1,2 dichlorobenzene
1,4 dichlorobenzene
chlorobenzene
pentachlorophenol
toluene
ethylbenzene
phenol
2,6 dinitrotoluene

Substituted One and Two Carbon Compounds

methylene chloride
dichloromethane
1,1 dichloroethane
1,1,1 trichloroethane
1,2 trans-dichloroethylene
vinyl chloride
trichloroethylene
tetrachloroethylene

Nitrosamines

N-nitroso-di-n-prophylamine
N-nitroso diphenylamine

Having selected the compounds for study, a rationale for establishing the loading rates was developed. As a reference loading rate (1X), the sludge nitrogen loading of 500 kg/ha/year was selected as an intensive utilization design criteria. From the extensive municipal sludge characterization of Sommers (1977), the nitrogen content was taken as 5% on a dry solids basis. These values translate into a dry sludge loading of 5 metric tons/ha/yr.

From the available priority pollutant data, the average total solids concentration was 57,000 mg/l for primary sludge (containing the higher organic constituent levels). The average concentration in wet sludge was determined for each organic compound. This organic compound concentration was combined with the dry solids concentration and nitrogen loading rate to determine the loading or soil level as follows:

$$\frac{500\ \text{kg N}}{\text{ha yr}} \times \frac{\text{kg TS}}{0.05\ \text{kg N}} \times \frac{1\ \text{sludge}}{0.057 \times 10^{6}\ \text{kg TS}} \times \frac{\text{ha.15 cm}}{1 \times 10^{6}\ \text{kg soil}}$$

$$\times \frac{0.28 \times 10^{-6}\ \text{kg DnBP}}{1\ \text{sludge}} = 0.049\ \text{ppm in soil of DnBP}$$

(di-*n*-butyl phthalate acid ester)

For the chemicals used, the above calculation is given under each section of this report for the respective chemical under investigation. Each investigation usually involved multiples of the reference rate (1X) calculated as above. These were typically 1X, 10X, and 100X. Control soil (Norfolk sandy loam) having no added organic priority pollutant is referred to as 0X.

EXPERIMENT LOCATION AND FACILITIES

The greenhouse and analytical facilities are co-located to the west (about one mile) of the North Carolina State University campus and at approximately 35.8°N latitude and 78.65°W longitude. Two greenhouses were in use over the course of the experiment. One is a classical glass greenhouse containing sectioned areas. The floor area is 90 m^2 with table area of 45 m^2. All outside walls have louvered windows and water curtains for humidity and temperature control. A roof fan is used for air flow. For winter operation, a heat system is present to maintain growing temperatures. Year round experimentation is facilitated by the addition of 12 metal halide mercury lamps (1,000 watt, Spero Co.) to extend the photoperiod. The lights were placed on timers, activated on about September 1st and deactivated on about May 31st, to achieve a minimum of 16 hours day length.

The second greenhouse is a rainbow design (Stuppy Greenhouses, Kansas, MO) consisting of structural steel pipe covered with two layers of Monsanto 602 greenhouse film. A floor area of 100 m^2 with 45 m^2 of metal mesh tables are available for experiments. A natural gas heating and forced air cooling systems are available for temperature control.

Floor gravel pits serve as drainage and maintenance of humidity. Similar 1,000 watt metal halide lamps (24) are in place and used as with the first greenhouse. Both facilities have easily accessible electricity and water. As a part of the greenhouse system in this location, there are 24 hour maintenance personnel to ensure against loss of power and other emergency conditions.

Table 3 provides a record of which experiments were conducted in the respective greenhouses. Dates of the experiments also reflect the portion of the annual cycle during which the crop growth occurred.

MATERIALS AND PROTOCOLS

CHEMICALS

The study of organic priority pollutants was undertaken primarily using ^{14}C labeled compounds. Utilizing experience in the agricultural chemicals field with isotopic materials and plant uptake, the ratio of 4,500 disintegrations per minute (dpm) per gram of soil was selected as the desired ^{14}C activity level. At 2.2 x 10^6 dpm/Curie(Ci), the radiolabeled loading is 2Ci per kg soil. With the amount of ^{14}C label per mole of chemical known for a given compound, the level of radiolabeled chemical (ppm of dry soil) was then specified in each experiment separately.

However, the total amount of chemical applied (radiolabeled plus nonradiolabeled) was dictated by the expected soil concentration from municipal sludge containing the organic priority pollutant. These loadings were previously described. The difference between the soil level of a specific organic compound due to municipal sludge and that acceptable for reasonable ^{14}C analysis sensitivity was made up with unlabeled chemical. Thus, there was a variety of labeled:unlabeled ratios in the organic chemical loaded to the soil. These ratios were used in calculating the actual concentration of the specific organic compounds since analysis was principally by liquid scintillation techniques. The discussion of the ^{14}C activity and purity of unlabeled chemicals is given in detail as a part of the respective discussion of individual chemical experiment.

EXPERIMENT CONTAINER

Polyethylene pots (Zarn, Inc) of the dimensions shown in Figure 2 were sufficiently large to allow full plant growth, but small enough to contain convenient volumes of soil for greenhouse operations. Pots contained ports near the bottom to allow drainage. By convention the active zone was considered to be the top 15 cm and so the study chemicals were only introduced into this upper region. The bottom 6 cm contained the same soil of the upper zone, but with no added organic chemical. In all the specific organic chemical experiments, aluminum foil was used to line the container on the inside to prevent plasticizer contamination of the soil.

TABLE 3. EXPERIMENTAL SCHEDULE AND GREENHOUSE USE

Experiment	Dates		Greenhouse Used
	Planting	Harvest	
Phthalic Acid (I)	9/26/80	1/1/81	Glass
DnOP, DEHP, DnBP (II)	11/10/80	5/15/81	Glass and Rainbow Plastic
DnBP (II), DEP, CDEP (III)	7/31/81	12/7/81	Rainbow Plastic
PNA(s) IV	8/17/81	11/30/81	Rainbow Plastic
Sludge and DnBP, ClB, pDCB	9/16/82	2/2/83	Rainbow Plastic
DnBP residue study	7/25/83	-	Rainbow Plastic

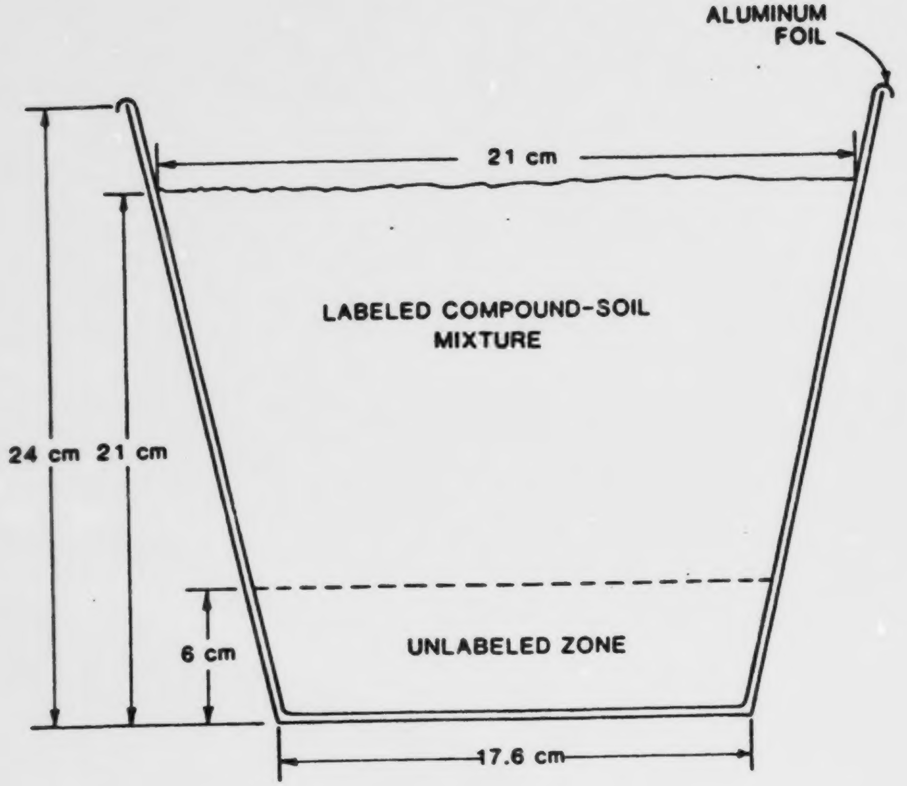
ALUMINUM FOIL
21 cm
LABELED COMPOUND-SOIL MIXTURE
24 cm
21 cm
UNLABELED ZONE
6 cm
17.6 cm

Figure 2. Experimental containers for crop and soil studies.

SOIL

The soil selection was made to obtain a common textural class. The Norfolk sandy loam was chosen. Since this soil has been used in over ten related environmental experiments concerning the fate of organic chemicals and wastes, the use in this study assists in comparison of results. A single soil was used in all studies so that soil type was not a major variable. Differences in batches of soil or from the aging process over the three years of this study (and ten years of other studies) lead to some variations in soil properties. These variations are not considered to be substantial or controlled.

A Norfolk sandy loam was chosen from a large top soil stockpile at the Central Crops Research Station, Clayton, NC. The soil is a fine, loamy, siliceous, thermic, typic, paleudult. Properties of the soil were determined repeatedly throughout the experimental program (Table 4). In the field the bulk density is 1.6-1.7 g/cm^3. However, without excessive work, it was only possible to compact the soil in these studies to a bulk density of 1.2. The field soil was air-dried, sieved through a 6.4 mm mesh screen, and stored indoors in a wood-lined soil bin. Prior to any experiment, the soil was wetted to field capacity and incubated for two weeks. This procedure has been found to allow an average level of soil microbial populations to develop prior to initiation of studies (Sims 1982).

TABLE 4. NORFOLK SANDY LOAM PROPERTIES

Soil Properties	Dates of Analysis					
	9/80	1/81	5/81	2/83	2/83	11/83
Organic Matter, %	1.3	1.7	1.3	-	-	-
Humic Matter, %	-	-	-	0.6	1.1	0.7
Particle Size, %						
sand	83	-	-	-	-	-
silt	7	-	-	-	-	-
clay	10	-	-	-	-	-
pH	6.1	5.9	4.8	5.2	4.7	4.9
Buffer acidity (pH 6.6)*	0.92	0.8	2	0.8	2	1.2
K*	0.09	0.07	0.08	0.8	1.0	0.05
Ca*	1.8	2	2	1.4	1.5	0.51
Mg*	0.74	0.6	0.52	0.22	0.42	0.21
Na*	L.D.L.***	0.1	0.06	-	-	-
Sum of K, Na, Ca, Mg	2.6	-	2.7	-	-	-
Base Saturation, %	-	77	-	75	-	-
Cation exchange capacity*	1.8	3.5	-	3.2	4.9	2.0
P**	8	10	23	190	>200	29
Mn**	4.8	3.8	5.6	2.4	6	3.4
Zn**	2.3	2.6	4.6	3.7	4.5	1.2
Cu**	1.8	1.2	1.8	1.1	0.9	0.68
Nitrate-N**	L.D.L.	-	15	-	60	1

* Extractable, meq/100 g

** Available, mg/dm^3

*** L.D.L.=less than detection limit

CONTAINER LOADING

Soil and Chemical

The entire pot to the height of 21 cm contained 7.8 kg (bulk density of 1.2 g/ml). After incubation at field capacity, the pot was inverted and carefully emptied onto a steel tray. The soil maintained the pot configuration and so the bottom 6 cm were removed and replaced back in the container. The remaining soil (5.8 kg) was mixed on a galvanized steel tray with a metal trowel with about 100 ml of solvent containing the organic chemical under investigation. This liquid-to-soil ratio was just sufficient to allow visual detection of soil wetting and thus to further aid in obtaining uniform chemical/soil mixtures. Since there are six containers for each loading rate of a single organic, a total volume of 600 ml of chemical in solvent are mixed. In later studies, a total volume of 750 ml was used to allow for some evaporative loss during mixing and to allow sample collection for analysis. Immediately after mixing the chemical and solvent with the soil, the 5.8 kg of treated soil is replaced in the containers at the 1.2 g/ml bulk density.

Number of Containers

For a single loading rate of one organic chemical, there are typically six pots. These include three replicates on which two plant species are seeded while on the other three replicates the other two plant species are placed.

Solvent Effects

Ethanol: This solvent was used to dissolve phthalic acid. In preliminary germination studies, three days for evaporative loss was found to still inhibit seed germination (Table 5). After four days, no visual or olfactory detection of ethanol was found and germination proceeded reliably.

Hexane: This solvent was found to have less effect on germination and was suitable as a carrier for the chemicals under investigation. Hexane was used in most of the germination/growth studies.

PLANT ESTABLISHMENT

Four different species were used as an experimental array to test the effects of various organic chemicals. These were:

- tall fescue
- corn
- soybeans
- wheat

Between plantings, all seeds were stored in bags in a laboratory refrigerator and thus used throughout the experiment.

TABLE 5. EFFECT OF SOLVENT ON SEED GERMINATION IN NORFOLK SOIL

Planting, days following solvent addition	Solvent Level, ml/460g Norfolk soil	Germination, %
Ethanol Solvent; Soybean Planting		
0	2.9	85
0	5.8	70
3	2.9	95
3	5.8	90
5	2.9	100
5	5.8	95
Hexane Solvent; Corn Seed Planting		
0	2.9	97
3	2.9	97
5	2.9	97

Five pounds of 'Kentucky 31' tall fescue (Festuca arundinacea) Schreb.) were obtained from FCX, Inc. (dated 1979) in July 1980. Tall fescue is native to Europe, and this particular variety was first collected by E. N. Fergus from William Suiter's farm in Menifee, Kentucky in 1931. It had apparently been growing there since 1887. It is adapted to a wide range of soil types, temperature extremes and is suited to the upper South.

Exhibiting medium relative maturity, the grass remains green all year except during mid-summer months and it is very productive, although not extremely palatable. It was released in 1942 by the Kentucky Experiment Station. The germination was rated as excellent.

Fifty pounds of 'Pioneer 3368A' Corn (Zea mays L.)(size F14, 95% germination, dated November 1979) were obtained from the North Carolina Crop Improvement Association, Inc. It is a modified single cross with outstanding yield for maturity. Disease tolerance, test weight and standability are also very good. Relative maturity is reached in 119 days (AES rating). It is closely related to Pioneer 3369A and is a modification. Pioneer 3368A possesses better leaf disease tolerance and tends to stay green longer in the fall than predecessor species.

Sixty pounds of Altona soybeans (Glycine max [L.] Merr.) (Lot R07990, 96% germination, dated 1979, tested November 1979) were obtained from the Minnesota Crop Improvement Association, St. Paul, Minnesota 55108. This variety originated from a cross between "Flambeau" and 052-903 at the Department of Plant Science, University of Manitoba. Altona is a Group 00 maturity and matures extremely quickly. Lodging resistance and seed quality scores are fair,with seed yield, seed weight, and seed protein and oil contents rated as good.

Thirty pounds of Butte wheat (Triticum aestiuum L.) (dated 1979) were obtained from Dr. R. C. Frohberg, Department of Agronomy, North Dakota State University, Fargo, ND 58102. The pedigree of this variety reads: ND480/Polk/Wisconsin 261. It is a hardened spring wheat, photoperiod insensitive, released in 1977 but never registered. It matures earlier than Waldron and has outstanding test weight.

Matching the container size, it was decided to allow only soybeans and wheat to grow through maturity with seed production. Corn grown to ear maturity was prohibitive for greenhouse studies while fescue was nearly mature after about 30 days. Thus, the arrangement in Figure 3 represents the plant combination used in these studies.

PRELIMINARY STUDIES

Four pots were grown to provide information on planting density, nutritional aspects, and management. Two pots were each planted to 10 seeds of corn (Zea mays L. 'Pioneer 3368A'), and 25 seeds of wheat (Triticum aestivum L. 'Roy'); the other two pots were planted to 15 seeds of soybean (Glycine max L. Merr. 'Ransom') and 10 ml of tall fescue seed (Festuca arundinacea Schreb. 'Kentucky 31'). Thirty ml of

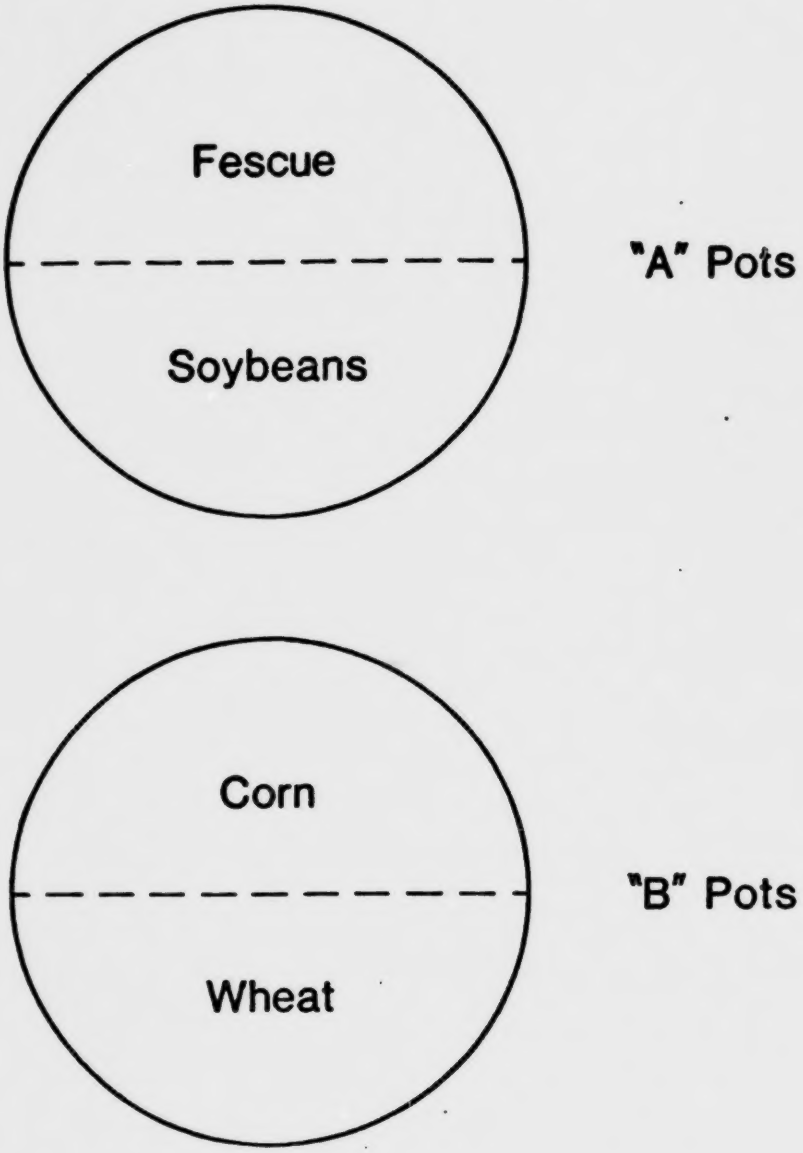
Fescue
Soybeans
"A" Pots
Corn
Wheat
"B" Pots

Figure 3. Experimental pot configuration of plants.

nutrient solution (Weber 1977) were added at planting and at weekly intervals.

As a result of this study, it was recommended that:

1. Corn be spaced more evenly, that 6 seeds be planted, and that harvest should be at the four leaf stage.

2. The number of wheat seeds be reduced to 20 and thinned to 10 plants after 18 to 20 cm of growth.

3. More care should be taken with the depth of soybean planting, and that 12 seed should be used and thinned to 6 or 7 plants.

4. The fescue stand was much too thick and the volume of seed should be reduced to 1.5 ml and spread over more of the pot surface.

5. The use of nutrient solution as fertilizer be abandoned. To provide adequate fertility, approximately 160 ml/pot/week would be required. To have a more uniform fertilizer availability, it was decided to use Osmocote^R (a timed-release fertilizer) at 1 Tbsp./pot every 3 months with additional NH_4NO_3, if necessary.

ROUTINE PLANTING AND GERMINATION PROTOCOLS

In vitro germination: On a periodic basis, the viability of seed that had been stored was measured under standard conditions. A fixed number of seeds were kept on a moist filter paper in a petri dish for about 7 days. At that time the emergence of the seeds was checked visually and the percent germination recorded. These results are given in Table 6 and reflect the variations in seed viability over the entire three year investigation.

Preparation and Planting: About two weeks before each experiment, a series of nutrient status containers were planted. Usually four pots, containing the four plant species at approximately the seed density used in the experiment, were begun. These were maintained as those of the experiment with regard to greenhouse conditions, water, and nutrients; and thus these pots allowed early detection of experimental problems and correction in the main experiment.

After the chemical loading and solvent vaporization, each pot received approximately 30 g of Osmocote^R 14-14-14 time release fertilizer (15 ml/pot). Earlier use of Osmocote^R with the solvent present led to dissolution of the Osmocote^R and corresponding adverse salt effects. In each section of the respective containers (Figure 3), the following numbers of seeds were planted in the shallow surface soil (upper 2 cm):

6-10 seeds - corn
20-30 seeds - wheat
12-20 seeds - soybeans
1.5-2.5 ml of seed - fescue

TABLE 6. GERMINATION OF SEEDS USED IN THREE-YEAR STUDY

Plant Species	Date of Emergence, %		
	3/13/81	7/21/81	7/25/83
Corn	100	100	100
Soybeans	100	90	90
Wheat	100	93	90
Fescue	81	92	60

There were some differences throughout the various experiments, but in each case the quantity of seeds actually planted were recorded to establish the germination percent.

MAINTENANCE OF EXPERIMENT

The glass greenhouse, equipped with humidification side screens and a roof fan, was used from Fall 1980 to Spring 1981. A continuous recording thermohygragraph was used to monitor the diurnal variations in temperature and relative humidity in the greenhouse. The typical range of maximum and minimum relative humidity was 50%-75% and 30%-50%, respectively (see Appendix 1 for representative daily values). Average daily maximum and minimum temperature ranges were 76^{O}F-90^{O}F and 60^{O}F-70^{O}F, respectively.

In December 1980, the polyethylene, air pressurized greenhouse was used for the experimental program. Better humidity and temperature control was achieved in this greenhouse unit. Average weekly maximum and minimum humidities and temperatures were 58-80%, 40-58%, 79-104^{O}F, and 58-81^{O}F, respectively.

The rigorous investigation of organic chemical leaching was not a major objective of this project. Thus, the addition of water was based on crop needs. Usually a daily observation was made of all pots and plant species to determine initial wilting and hence the need for watering. Water was added with a hose, if necessary, with all pots receiving approximately the same timing and volume. Osmocote^{R} was added to the surface of all pots every 3 months. On occasion the nutrient status pots indicated the need for fertilizer and a small amount of NH_4NO_3 was added.

Insect pests were controlled with oxamyl (Vydate; methyl N^1, N^1 - dimethyl -N- [(methyl carbomoyl) oxy] - 1 thiooxaminidate) and diazinon (Spectracide; 0,0-diethyl-0-(2 isopropyl -4-methyl -6-pyrminidinyl) phosphorothioate). Disease pests were controlled with benomyl (Benlate; methyl - 1 - (butylcarbamoyl) - 2 - benzimidazolecarbamate). Cyhexatin (tricyclohexyl hydroxystannane) was used to control spider mites on soybean plants when necessary.

In the operation of the specific organic chemical experiments, difficulties in maintaining plant viability were encountered. These were typically in the very early stages when germination and emergence were occurring. When extended cloudiness occurred immediately after seed planting, the majority of the seeds did poorly and sometimes replanting was necessary. After the early growth stages, few difficulties were encountered in maintaining viable plants. Standard crop maintenance procedures were generally used throughout these investigations (Weber 1977).

HARVESTING

General criteria were used in all experiments to determine when to harvest the various plant species. The crops did not have the same tolerances for warm and cool seasons nor the same photoperiod response. Since studies were initiated at many different times during an annual cycle, the elapsed number of days to harvest varied among experiments.

As a general description, the corn was harvested substantially before maturity. Fescue was also harvested before seed formation, but closer to the typical agricultural height as cut in the field. Both corn and fescue were completely removed from the respective pots with the harvesting. The roots were left in place and appeared to decompose during the remainder of the experiment. At about the same time, the other two species, soybeans and wheat, were sampled and thinned to allow the remaining plants to grow to maturity with production of seeds. Since the amount of the specific organic chemical taken up in the above ground plant is routinely a small percent of the amount applied to the pot soil, the staggered removal of crops had little effect on the presence of the chemicals for further uptake or other fate processes.

The criteria for harvesting or sampling the various crops are as follows:

1. Corn at the 4-leaf stage, approximately 60 cm height
2. Fescue at 20-25 cm height
3. Wheat thinned at about 20 cm height, harvested at about 2 weeks after dough stage
4. Soybeans thinned at approximately 25 cm tall and harvested when the plants are senescent after sufficient seeds have formed.

All plant and seed samples were prepared and placed in paper bags and then refrigerated until analysis. Actual harvest, expressed as elapsed time from successful seed germination, varied among experiments.

EXPERIMENT TERMINATION

Following the vegetation harvest, the pots contained soil and roots. A sample was taken of the surface 15 cm to which the organic chemical had been originally added. Each container was then inverted and carefully emptied using the aluminum foil to withdraw the soil intact. The bottom zone was then sectioned and a sample of the soil (which did not originally contain the organic chemical) was taken. Next the roots that could be routinely removed were taken by hand from the remaining soil.

SAMPLING AND VERIFICATION USED IN EXPERIMENTS

In the following sections, the variety of routine procedures that were common to most of the individual experiments on specific organic compounds are discussed.. These descriptions also serve to define the nomenclature used in reporting the data of each experiment.

SOIL LOADING VERIFICATION

In routine studies, a mixture of radio-labeled and unlabeled organic chemical was added to a solvent and then put on the soil. A table was prepared of the volume of the labeled and unlabeled chemical (often already dissolved in a small volume of solvent and at different concentrations) to be added to the large solvent volume (600-750 ml). The entries in this table were followed to achieve the serial chemical application rates that were the subject of these studies. The initial verification of the anticipated soil loadings was obtained by sampling the solvent/chemical solution prior to addition on soil. The resultant chemical concentration and hence mass of specific compound were used as the actual loading rates or initial soil concentration of a specific chemical.

As a further loading verification in selected experiments, a sample of the soil was taken after mixing with the solvent/chemical solution but before placement back into the container. Then using the extraction procedure described below, the initial soil concentration was determined to define the relationship to crop uptake.

SOIL AND CROP TESTS FOR EXTRACTION EFFICIENCY

On each chemical compound studied, the extraction efficiency was established as a routine experimental procedure. Chemical was introduced into plants, by injection, and onto soil and then the extraction procedure undertaken. The extraction method varied with chemical and so is described in those sections. The percent extractability was then calculated for each compound, and used to assess the methodology used.

A Harvey Oxidizer was used to determine total ^{14}C, and the extraction efficiency was determined on crops and soils that had been used throughout an experiment. In this manner, any processes which could alter extraction efficiency over the length of the experiment was assessed. This sample extraction efficiency was then used to correct extractant scintillation results for the efficiency of that extraction. Results for specific chemicals and crops are given in the respective chapters of this report.

SOIL SAMPLING AT END OF EXPERIMENT

Having removed the aboveground portion of all vegetation, the pots were sampled to allow determination of the remaining organic compound. The first sample is that of the soil which originally received the loading of each chemical. A corer (1.5 cm diameter, 11 cm in length) was inserted fully through the upper surface of the soil. The concentration determined for this sample was used in the mass balances to assess the entire upper 15 cm of soil. In this manner, the soil corer did not penetrate and contaminate the lower 6 cm zone (originally without chemical). The bottom 4 cm was sectioned from the inverted container soil contents and analyzed for the study chemical. The resulting concentration was used to represent the entire lower 6 cm of soil in the mass balance determinations. All chemical concentrations are expressed on a dry soil basis. The moisture determination for these calculations were done by weighing a soil aliquot before and after an overnight period kept in an oven at 100°C.

CROP SAMPLING FOR ROUTINE ANALYSES

For all aboveground vegetation samples, the resultant fresh material was sectioned into pieces 1-2 cm in length. The entire sample was weighed immediately. From the sample, a 4-8 g subsample was taken for moisture determination. The subsample was dried at 70°C to constant weight over about 7 days. The remaining fresh material was placed in paper bags and frozen until ready for analysis.

The exception to the above preparation procedure was for the seed portion of mature soybeans and wheat. These seeds were collected from the plant by hand. Soybean pods were opened manually and the seed removed for analysis. The pods were combined with the rest of the nonedible aboveground plant material for subsequent preparation. Wheat seeds were likewise separated from the chaff manually and analyzed as a separate, edible fraction of the plant.

CROP EVALUATION OF ROOT:SHOOT RATIO

In determining a mass balance on a given chemical, the soil fraction had to be separated into two parts, roots and soil. A representative sample in the entire pot of roots and soil together was unobtainable, particularly since it was desirable to separate soil into the upper 15 cm and lower 6 cm zones. Thus, the mass of roots had to be determined. With the negligible differences in plant growth at various chemical loadings, the roots were assumed to also be uniform in mass. Further, the roots were assumed to be proportional to the mass of aboveground fresh weight. Under these assumptions, the root mass was compared to the aboveground mass (plant, seed, and weeds) to determine a root:shoot ratio. This ratio was determined only for mature soybeans and wheat since these values are the only ones used in the final mass balance of each chemical. By maturity the roots of corn, fescue, thinned soybeans, and thinned wheat had by visual observation been mineralized. Table 7 contains the root:shoot ratio measurements made during these studies. The levels of a specific chemical found in the roots of plants (ppm on fresh root basis) were then converted into the total amount of chemical in the roots as follows:

TABLE 7. ROOT:SHOOT RATIOS AT EXPERIMENT TERMINATION

Wheat & Corn Pots		Fresh Weight Ratios	Dry Weight Ratios
EPA IV			
Benz(a)anthracene			
	0X	5.02	5.25
	1X	3.37	5.58
	10X	2.05	3.09
	100X	1.65	2.46
	Avg.	3.02	4.10
EPA III			
DnBP	0X	4.44	4.80
	1X	5.94	6.63
	10X	5.60	5.77
	100X	6.09	6.22
	Avg.	5.52	5.85

total organic chemical mass in roots at maturity = (ppm on fresh root basis) for each pot x (shoot:root fresh weight ratio) determined on a few pots x (fresh mass of mature plant) at final harvest for each pot

The resulting values were combined with the chemical content of the soil sampled directly to yield the total mass of a particular chemical remaining in the container after the final harvest.

CROP STUDIES OF CHEMICAL TRANSLOCATION

The process elucidation by which a specific organic chemical might migrate from the soil to the edible seed portion of wheat or soybean were not a major focus of this project. However, some effort was devoted to this translocation issue. Using both wheat and soybean, a limited amount of the organic chemical was injected directly into the plant stem just after the start of seed growth. For wheat, the start of the seed head was noted by feeling the seed segment of the plant. For soybean, an arbitrary 1-2 weeks after flowering was used as a signal of seed initiation. Isolated control plants in pots not having chemical addition were used for these studies so that chemical translocation from the roots would not be occurring in parallel. The plants were isolated by transplanting to separate pots with control soil so that manipulations would not accidentally contaminate the main experimental control plants. Several chemicals were studied with this technique. The hypothesis tested was that the chemical, if present, could move to the seed during the period of substantial seed growth.

After these injected plants were grown to maturity, the seeds were harvested and analyzed using the standard protocol. The plant plus pods or chaff were harvested and analyzed so that recovery of total injected chemical as well as that appearing in the seed could be determined. These results are presented elsewhere in relation to the behavior of each specific chemical.

CROP EVALUATIONS FOR CONSISTENCY OF SAMPLING AND ANALYSIS

As an aid for technique quality control, separate tests were made of the sampling and extraction protocol. One day prior to the aboveground vegetation sampling (of all species), a known quantity of chemical was injected into an isolated control plant. This plant was then harvested and treated as a routine sample. The results when compared to the previously established extraction and recovery efficiencies allow for corroboration that any particular sampling/analysis event and resulting data were routine.

CROP STUDIES TO VERIFY PRESENCE OF PARENT COMPOUNDS

The basis for determining crop uptake or the presence in soil of a number of specific chemicals in these experiments is ^{14}C detection. The ^{14}C is originally a part of a study chemical such that the liquid scintillation results (dpm) can be related to the mass of parent compound. While this technique has the advantage of very low levels of detection, the disadvantage is that detected ^{14}C may not be the parent compound. That is, after accounting for natural background levels, the ^{14}C detected could still be a metabolite of the parent compound; hence, some ambiguity results. In an active biological system such as the soil and with degradable organic compounds, it is likely that detected ^{14}C could be parent compound, metabolite, newly formed chemical, a part of biomass, or any combination thereof.

In order to clarify these possible results, the verification of the detected ^{14}C as parent compound is needed. Throughout these experiments, attempts were made to achieve this verification. A variety of analytical techniques were selected as appropriate for the particular organic chemical present. In almost all cases, plants were selected that had high levels of ^{14}C, usually corresponding to the highest soil loading rate. The assumption made was that the mechanism for uptake was the same over the range of soil loading rates used for a particular chemical. The selection of only the high levels of ^{14}C was necessary because of the inherently lower limits of detection for the verification analytical techniques.

SUMMARY OF SAMPLE NOMENCLATURE

With the variety of principal and secondary studies that were a part of these experiments, it is useful to summarize the nomenclature used in reporting results.

- background - the ^{14}C or specific chemical content of the solvent used to extract the parent compound when that solvent has been put through the standard extraction procedure;
- natural vegetation (or control) - the ^{14}C or specific chemical content of individual crop species when grown, sampled, and analyzed under the routine experimental conditions but with soil containing no added organic chemical;
- vegetation analysis - the crop species grown under the routine experimental conditions with soil amended by addition of varying levels of specific organic chemicals;
- translocation analyses - results from experiments on natural vegetation which has been injected with specific chemicals into the stem just after initiation of seed growth to determine whether, if present, the chemical would move to the seed during the period of signifiant seed growth.
- quality control results - the corroboration of sampling, handling, and analysis techniques by injecting known quantities of a specific chemical into the plant just before harvesting to determine whether reasonably uniform results were achieved during multiple experiments.

- control soil - analysis of ^{14}C or specific organic chemical from soil placed in containers but receiving no direct chemical input;
- amended soil - the control soil to which specific organic chemical, usually as a mixture of ^{14}C labeled and unlabeled compound, has been added.
- root mass - tne below ground portion of mature wheat or soybean that is estimated for individual containers from the root:shoot ratio and the measured aboveground fresh weight;
- surface soil - the soil upper 15 cm of each container to which a specific organic chemical has been uniformly mixed;
- leach zone (bottom soil) - all soil (usually the lower 6 cm in the pots) except the surface soil which as the lower zone receives specific organic chemical only by migration from the upper zone.

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SECTION 5

CROP PLANT GROWTH, UPTAKE AND SOIL RETENTION OF PHTHALIC ACID

INTRODUCTION

Application of municipal and industrial sludge to agricultural land has become common practice. Past research has examined the effect of heavy metals in sludge on crop plant uptake and growth. Recently, concern has been expressed about plant uptake of toxic organic compounds from sludge (Gonzalez-Villa et al. 1982). Shea et al. (1982) reported that low levels of di-*n*-butyl phthalate were taken up from soil by corn plants.

Phthalic acid (1,2-benzene dicarboxylic acid) consists of a benzene ring with two adjacent (ortho) carboxylic acid groups attached. It has a density of 1.593 gm/ml and has relatively high water solubilities of 0.54 gm/100 ml at 14°C and 1.01 gm/100 ml at 35°C (Seidell 1928). Phthalic acid has a rather high acute oral toxicity (LD_{50}) of 12.6 gm/kg in rats (Leah 1977). Verschueren (1977) cited a LD_{50} of 3.2 gm/kg in rats. For comparison, rat acute oral toxicities (LD_{50}) of some phthalic acid esters can be similar (di-*n*-butyl phthalate, 12.5 gm/kg), higher (di-2-ethyl hexyl phthalate, 30.6 gm/kg) or lower (dimethyl phthalate, 6.8 gm/kg) (Leah 1977).

Phthalic acid is used to tan hides and assist in the removal of Cr from tannery wastes (Balberova et al. 1983). It is also used as a catalyst in resorcinol-formaldehyde resin manufacturing (Gamidov et al. 1982) and as a corrosion inhibitor (Nakajima et al. 1969). Phthalic acid forms Fe chelates and can be used to remove pyritic Fe from coal (Burk et al. 1979). It is found in waste from the polyester fiber industry (Leenheer et al. 1976). Jolley et al. (1976) found 200 ppb of phthalic acid in primary domestic sewage effluent. Raikina et al. (1980) reported phthalic acid concentrations of 0.027 to 1.34 gm/lit in wastewater from a dialkyl phthalate manufacturing plant in Russia. The U.S.S.R. has set a limit of 0.5 mg/lit to prevent inhibition of self-purification processes in receiving water (Verschueren 1977).

The phthalic acid esters (particularly di-ethyl hexyl, di-isodecyl, di-ethyl, and di-butyl phthalates) are industrial plasticizers commonly found as low level environmental pollutants (Peakall 1975). Phthalic acid is a common microbial breakdown product of phthalates in freshwater (Sodergren 1982, Saeger and Tucker 1976), activated sludge (Saeger and Tucker 1976, Shelton et al. 1984), and under anaerobic conditions (Benckiser and Ottow 1982, Shelton et al. 1984). Shelton et al. (1984) found that anaerobic breakdown of butyl benzyl phthalate results in a peak accumulation of phthalic acid at 10 days with a gradual decline to zero by 24 days. Phthalic acid is also present in low levels in precipitation. Likens et al. (1983) detected a variety of organic acids in precipitation in Ithaca, N.Y. and Hubbard Brook (BH) Experimental Forest, N.H. They found phthalic acid in samples from HB at less than 0.1 umol. Other organic acids (e.g., acetic acid at 2.8 umol at HB and

1.1 umol at Ithaca; formic acid at 0.2 umol at HB and 2.4 umol at Ithaca) were present in higher concentrations. Matsumoto and Hanya (1980) also found phthalic acid in atmospheric fallout near Tokyo, Japan.

Degradation of phthalic acid is rapid in soil. Conversion to benzoic acid by Bascillus sp. and Pseudomonas testosteroni was reported by Taylor and Ribbons (1983). Afting et al. (1981), Harada and Koiwa (1977) and Englehardt et al. (1976) isolated various soil bacteria which rapidly degraded phthalic acid to protocatechuate. El-Shinnawi and Shalabi (1981) studied soil degradation of 16 organic acids and reported that citric and phthalic acid degraded more slowly than other organic acids. The input of phthalic acid to agricultural land from the atmosphere is probably minor. More important vectors are directly from sewage sludge and from phthalate ester breakdown in the soil.

MATERIALS AND METHODS

The procedures common to most of the experiments conducted in this research are described in the previous report section.

CHEMICALS

^{14}C-labeled phthalic acid (Amersham CFA 766, Batch 5, Specific Activity 60 mCi/mmol, 6400 dpm/gdw soil) was added to unlabeled phthalic acid (Fisher certified grade) in ethanol and thoroughly mixed into the upper 12 cm (5.8 kg) of the soil in each pot. Three replicates of a randomized, complete block design were used with loading rates of 0.6, 6.0, 60.0, and 600.0 ppm and two crop combinations. Six control pots were also included for a total of 30 pots.

CROP PLANTS

Seeds were planted on September 26, 1980. Poor germination necessitated replanting on October 20, 1980. Height measurements were made and recorded weekly. Corn and fescue plants were harvested at 21 and 45 days after the final planting, respectively. Soybean plants were thinned and retained for analysis at 21 days and the remaining plants were harvested when mature at 74 days. Wheat plants were transplanted from untreated pots to make six wheat plants available for measurements of mature growth and chemical uptake. Wheat plants were thinned at 40 days and remaining plants harvested at 74 days. Wheat and soybean seeds were separated by hand from nonedible plant portions. Weeds were also harvested and retained for analysis when the final crop was sampled.

CHEMICAL ANALYSIS

Plant samples were homogenized for one minute with a "45" Virtis homogenizer at about 30,000 rpm. Fifty ml of hexane:acetone (1:1 Fisher H-300 pesticide grade and Fisher A-18 certified grade, respectively) were used for each sample. After homogenization, the blades were washed into the grinding flasks and flask contents were filtered through a Whatman GF/A 5.5 mm glass microfiber filter under suction into a side-armed Erlenmeyer flask. This flask was washed with hexane:acetone into a 150 ml beaker and the filtrate evaporated to dryness under a hood. The filtrate was redissolved in five ml of methanol, ultrasonicated for one minute, and two ml were removed and retained for later analysis. Several drops of sodium hypochlorite bleach (Chlorox^R) were added to the vials to bleach out the chlorophyll in the remaining three ml of filtrate. Vials were placed under fluorescent lighting for 15 minutes to 2 days to aid in chlorophyll bleaching.

Scintillation cocktail [16.5 gm 2.5-diphenyloxazole (PPO), 0.5 gm 1,4-bis[2-(4-methyl-5-phenyloxozoyl)] benzene (POPOP), 1000 ml Triton X-100 and 2000 ml toluene] was added to each vial. A liquid scintillation spectrometer (Packard Tri-Carb Model 2405) was used to twice assay each vial (20 minute counts). Observed radioactivity was corrected for counting efficiency which ranged from 77 to 89%.

In soil extractions, a 50 gram soil sample was weighed, placed in a plastic beaker with 50 ml of hexane:acetone (1:1) and ground for two minutes with a tissue homogenizer (Tek-Mar Homogenizer). After settling for 30 minutes, the sample was filtered under suction through Whatman GF/B Glass Microfiber filter paper into a side-armed Erlenmeyer flask. The filtrate was allowed to evaporate until nearly dry; contents were then transferred to a scintillation vial with 1:1 hexane:acetone washes and then allowed to dry overnight. Four ml of methanol were added to each vial, allowed to stand overnight, and ultrasonicated for one minute. Two ml were retained for later analysis and two ml were radioassayed using the same methods as described previously.

EXTRACTION EFFICIENCIES

A mixture of ^{14}C-labeled and unlabeled phthalic acid was injected into mature corn and soybean stems with a microliter syringe. After 24 hours, the plants were harvested and analyzed to determine extraction efficiencies. The ^{14}C-phthalic acid mixture was mixed into soil samples in an Erlenmeyer flask, extracted and analyzed after two hours. For both plant and soil samples, ^{14}C chemical injection levels were calculated to be similar to those found in extracted samples. Chemical extraction efficiencies averaged 75% for corn and soybean plants and 87% for soil.

PLANT UPTAKE AND SOIL CONCENTRATION CALCULATIONS

Concentrations of phthalic acid were calculated by subtracting background counts (from control samples); multiplying by 1.5 or 2.0 (to account for sample splitting for plant and soil samples, respectively) and dividing by sample dry weight to yield ^{14}C activity as disintegrations per minute per gram of fresh weight (dpm/gfw). A conversion factor was calculated acid [(mg ^{14}C-phthalic acid X dpm/mg ^{14}C-phthalic acid)/ (mg ^{14}C-phthalic acid + ^{12}C-phthalic acid)]. The inverse of this value when multiplied by sample dpm/gfw yielded ppm of ^{14}C in the sample. This value was divided by chemical extraction efficiency (75% for plants and 87% for soil) and then divided by percent dry weight to yield ppm ^{14}C dry weight. This value was multiplied by 1000 and by the percentage of radiation identified as phthalic acid by TLC analysis to yield parts per billion phthalic acid.

Total harvest fresh weight for each crop was multiplied by crop dpm/gfw for each pot and divided by ^{14}C extraction efficiency determined from oxidation. These values were summed for all plants in each pot to determine total aboveground ^{14}C in each pot. ^{14}C extraction efficiency values were not obtainable for wheat and soybean seed; the values for mature wheat and mature soybean plants were used. Total radiation in bottom (5 cm) and top (12 cm) soil samples was determined in the same manner as plants. The middle 3 cm of soil was undisturbed and assumed to have top soil ^{14}C concentrations. To estimate root biomass, all roots were removed from the pots by sieving the soil through two soil sieves (4.00 mm and 1.7 mm). Roots were weighed, a subsample dried for dry weight determination and the remainder frozen for later chemical analysis. Root:shoot ratios were calculated. Root ^{14}C extraction efficiency was assumed to be the same as the surrounding soil. Total dpm found in aboveground plants, roots and soil were summed and chemical extraction efficiencies included. Total dpm accounted for were divided by the number of dpm originally applied to calculate percent recovery.

Determination of unextractable (bound) ^{14}C was done for plant and soil samples after extraction by oxidation in a Harvey Biological Oxidizer (OX-300) for 4 minutes under O_2 and N_2. Released $^{14}CO_2$ was captured in Harvey Cocktail and samples were counted as before. Insufficient samples were available for analysis of seeds, and so mature plant ^{14}C extraction efficiency values were used.

THIN LAYER CHROMATOGRAPHY (TLC)

To determine the percentage of extractable ^{14}C which was phthalic acid, a 2 ml aliquot of duplicate extractions was streaked onto 1000 um silica gel plates (Type GF, Sigma Chemical Company). Plates were developed in benzene:acetic acid (8:2). The phthalic acid rf value was determined using standards mixed with control sample extract and developed in a spark chamber (Birchover Inst. Co.; 9.84% methane in argon). Plates were sectioned, scraped and counted. The percent of the total ^{14}C on the plate which was located at the phthalic acid rf was determined.

STATISTICAL ANALYSIS

Data were generated with the General Linear Model (GLM) program from SAS. Plant and soil retention data were transformed to $\log_{10}$ after converting all data to X+1 for the GLM program to calculate analyses of variance. Variables used in the ANOVAS were replicate and rate. For fescue, only rate was used in order to retain sufficient degrees of freedom. Regressions were done to predict phthalic acid uptake based on original soil loading rate; the SAS STEPWISE program was used.

RESULTS

PLANT GERMINATION AND GROWTH

Wheat germination was reduced by high rates of phthalic acid. Corn and soybean germination were not affected (Table 8). Similarly, the number of corn and mature soybean plants at harvest was not affected by phthalic acid (Table 9). The number of immature soybeans was apparently enhanced by phthalic acid; however, this was probably caused by unusually poor soybean germination in control pots. The number of mature wheat plants at harvest was apparently enhanced by phthalic acid. This reflects plants transplanted into these pots after the first harvest. There was no significant effect ($p \leq 0.05$) of rate or replicate on plant dry weight or height except for immature wheat height (Table 10). This increased at 600 ppm rate.

PLANT UPTAKE

The percent of extractable ^{14}C which was identified by TLC as phthalic acid was 4.5% for corn, 5.2% for fescue, 9.2% for mature wheat, 15.0% for mature soybeans and 46.7% for wheat seed. Sufficient samples were not available for TLC work on immature wheat and soybean plants, or soybean seed. Average percentages for similar aged plants were used in the conversion of extractable ^{14}C to extractable phthalic acid. The percentages of ^{14}C identified as phthalic acid were assigned as follows: immature wheat and soybean plants at 4.8% and soybean seed at 46.7%. Average uptake of extractable phthalic acid ranged from zero to 23 ppm (Table 11). Uptake was significant ($p \leq 0.01$) for all plants except soybean pods. Fescue and immature wheat plants had the greatest uptake while soybean pods and wheat seed had the least. Uptake was significant only at the 600 ppm rate for all plants except soybean pods. Fescue uptake was significant at the 6.0, 60 and 600 ppm loading rates.

Predictions of plant uptake of phthalic acid were made from linear regression models (Table 12). The best regressions were based on soil loading rate with r^2 ranging from 0.79 (immature wheat plants) to 1.00 (fescue). When data from all immature plants, all plants or all seeds were analyzed together, regression accuracy dropped considerably to 0.60, 0.51, and 0.63, respectively. A regression with the two mature plants (wheat and soybean) was still quite accurate (r^2 of 0.93). These models can be used to predict phthalic acid uptake (ppb dry weight) based on soil loading levels of phthalic acid (ppm dry weight). Extraction efficiencies (^{14}C) in plants were 38.1% (corn), 19.2% (mature wheat), 24.9% (fescue), 31.4% (immature soybean plants), 39.5% (mature soybean plants), and 43.4% (wheat seed). These values were used in the ^{14}C balance calculations. No values were obtainable for immature wheat or soybean seed. They were assumed to have 43.4% and 39.5% ^{14}C extraction efficiencies, respectively.

TABLE 8. PERCENT GERMINATION OF PLANTS GROWN IN GREENHOUSE POTS TREATED WITH PHTHALIC ACID

Rate, ppm	Corn	Soybean	Wheat
0	63	82	57
0.6	63	69	59
6.0	60	71	59
60.0	60	80	36*
600.0	70	80	44*
Statistics			
F	0.70	0.26	4.63*
L.S.D. (t at .05)	-	-	5.3

*Statistically significant at $p \leq 0.05$.

TABLE 9. NUMBER AND STANDARD DEVIATION OF PLANTS AT HARVEST IN GREENHOUSE POTS TREATED WITH PHTHALIC ACID

Rate, ppm	Corn	Immature Soybean	Mature Soybean	Immature Wheat	Mature Wheat
Planted	10	15	15	25	25
0	6(0)	7(1)	6(1)	8(2)	6(2)
0.6	6(1)	10(2)*	6(0)	10(1)	7(0)
6.0	6(1)	10(2)*	6(1)	9(2)	6(1)
60.0	6(1)	12(2)*	6(0)	3(2)*	6(0)[a]
600.0	7(1)	6(0)	6(0)	5(0)	9(3)[a]
Statistics:					
F	0.73	6.16*	0.59	5.69*	2.71**
L.S.D. (t at 0.05)	-	3.0	-	4.5	2.5

[a] Plants transplanted from control pots to treated pots after thinning.

* Statistically significant at $p<0.05$.

** Statistically significant at $p\leq0.10$.

TABLE 10

DRY WEIGHT (GM), HEIGHT (CM) AND STANDARD DEVIATION OF PLANTS GROWN IN GREENHOUSE POTS TREATED WITH PHTHALIC ACID

Rate ppm	Corn	Fescue	Immature Soybean Plants	Mature Soybean Plants	Soybean Seeds	Soybean Pods	Immature Wheat Plants	Mature Wheat Plants	Wheat Seeds
Dry Weight									
0	3.2(1.0)	1.4(0.6)	1.2(0.8)	8.1(2.9)	9.6(2.6)	13.0(3.2)	0.7	23.1(8.8)	5.1(1.7)
0.6	3.2(0.7)	1.5(1.1)	1.0(0.4)	6.8(0.8)	9.6(0.7)	13.0(0.8)	0.8(0.8)	21.1(1.4)	6.3(1.6)
6.0	2.6(0.8)	0.8(0.5)	1.3(0.5)	8.2(2.4)	10.8(1.7)	14.6(2.2)	0.4(0.3)	20.2(3.8)	4.7(0.4)
60.0	3.0(0.5)	1.0(0.8)	2.1(0.8)	8.6(2.1)	12.4(1.5)	16.6(2.2)	-	25.4(8.2)	5.6(2.3)
600.0	2.8(0.9)	1.9(0.3)	1.2(0.2)	7.5(1.9)	8.1(1.0)	10.8(1.5)	0.9(0.9)	28.4(5.6)	5.8(2.3)
Statistics:									
Ave(S.D.)	2.9(0.7)	0.8(1.3)	1.4(0.6)	7.8(1.9)	10.2(1.7)	13.6(2.0)	0.7(0.8)	23.6(5.6)	5.5(1.3)
L.S.D. (t at .05)	1.4	1.5	1.1	2.9	2.9	3.6	1.8	10.6	2.4
F	0.9	0.8	1.4	1.4	1.8	3.4	0.2	1.4	2.5
Height									
0	42(5.0)	20(6.4)	25(0.6)	40(2.1)	-	-	25(4.6)	58(4.9)	-
0.6	40(1.25)	20(1.2)	23(0.6)	41(3.2)	-	-	30(4.1)	47(2.0)	-
6.0	40(3.6)	20(3.6)	22(0.6)	46(4.4)	-	-	32(2.1)	52(3.6)	-
60.0	41(8.1)	22(1.5)	22(2.1)	48(4.5)	-	-	38(6.8)	50(3.8)	-
600.0	37(3.6)	19(1.5)	24(2.5)	48(7.5)	-	-	22(0.6)	54(3.2)	-
Statistics:									
Ave(S.D.)	40(4.3)	20(3.4)	23(1.3)	45(4.4)	-	-	29(3.7)	52(3.7)	-
L.S.D. (t at .05)	8.0	6.3	2.4	8.3	-	-	7.0	6.9	-
F	1.2	0.7	2.5	1.9	-	-	5.9*	2.8	-

TABLE 11

UPTAKE AND STANDARD DEVIATION OF EXTRACTABLE PHTHALIC ACID IN GREENHOUSE-GROWN PLANTS
ppb-dry weight

Rate ppm	Fescue	Immature Soybean Plants	Mature Soybean Plants	Soybean Pods	Soybean Seeds
0	0	0	0	0	0
0.6	23(19)	4(3)	3(1)	0(0)	6(3)
6.0	97(17)*	35(7)	47(18)	0(0)	42(36)
60.0	1359(.)*	1362(2)*	307(210)	0(0)	485(601)
600.0	23365(.)*	2842(534)*	4151(1306)*	0(0)	6119(2166)*
Statistics: F	59.0*	172.0**	155.2**	1.0	8.9**
L.S.D.	72	392	898	0.1	1900

Rate ppm	Corn	Immature Wheat Plants	Mature Wheat Plants	Wheat Seeds
0	0	0	0	0
0.6	2(1)	14(4)	1(1)	0(0)
6.0	56(11)	149(34)	12(6)	3(2)
60.0	532(233)	n.d.	7(4)	9(2)
600.0	6554(1069)*	14976(7583)*	3407(622)*	1711(917)*
Statistics: F	400.5**	95.7**	169.8**	126.8**
L.S.D.	1281	12581	436	820

n.d. no data
* Statistically significant at $p \leq 0.05$.
** Statistically significant at $p \leq 0.10$.
Data log transformed after changing all data to X + 1.
(.) Only one plant remaining.

TABLE 12

REGRESSIONS OF PLANT UPTAKE AND SOIL RETENTION OF PHTHALIC ACID

Species	A	B	r^2	F*
Corn	10.9	-30.8	0.98	393.5
Fescue	38.9	-146.7	1.00	4089.8
Immature Soybean Plants	4.7	15.4	0.98	489.7
Mature Soybean Plants	6.9	-33.6	0.92	180.6
Soybean Seeds	10.2	-34.7	0.89	110.8
Immature Wheat Plants	24.8	-0.3	0.79	27.0
Mature Wheat Plants	5.7	-67.5	0.96	441.0
Wheat Seeds	2.9	-44.9	0.80	47.0
Immature Plants	17.3	-62.4	0.60	60.7
Mature Plants	6.3	-48.5	0.93	422.8
All Plants	12.9	-82.3	0.51	81.5
All Seeds	6.5	-38.6	0.63	46.0
Top Soil	0.39	12.62	0.87	88.6
Bottom Soil	0.19	-3.35	0.58	18.1

Model Uptake (ppb dry weight-phthalic acid) = A (soil loading rate-ppm) + B
* all $p \leq 0.01$

SOIL RETENTION

Statistically significant amounts of phthalic acid remained in the soil for both sets of pots for all chemicals (Table 13). Amounts were significant at all rates except the 0.6 ppm rate.

A small, but significant, portion of the originally applied phthalic acid was leached into the bottom of the pots at all rates except 0.6 ppm (corn and wheat pots). An average of 0.02% of the originally applied chemical was recovered in the bottom. Some of the chemical may have leached out of the bottom of the pot but this is minor since pots were never overwatered.

Based on analysis of the ^{14}C balance sheets, most of the phthalic acid had been volatilized or decomposed by the end of the experiment (80 days). An average of 5.7% of the originally applied ^{14}C was recovered at the end of the experiment. A small portion of the recovered ^{14}C was found in aboveground plant tissue (0.3%).

Regression equations were produced to predict soil concentrations of phthalic acid at plant harvest (80 days)(Table 12). Both top and bottom soil equations were statistically significant ($p \leq 0.05$) but the top soil equation had a better r^2 than the bottom soil equation (0.87 vs. 0.58, respectively).

^{14}C extraction efficiencies were low for both sets of pots for top (10.2%) and bottom soils (5.7%). 24 hour Soxhlet extraction in methanol only increased the ^{14}C extraction efficiency to 13.6%.

The percent of extractable ^{14}C which was identified by TLC as phthalic acid averaged 20.0% for top soil and 13.8% for bottom soil. These values were used to convert extractable ^{14}C to extractable phthalic acid shown in Table 13.

CONCLUSIONS

Phthalic acid had little effect on crop plant height or dry weight up to a soil loading rate of 600 ppm. Immature plants generally had the highest uptake of phthalic acid which reflects higher soil levels while plants were young. Mature wheat plants and wheat seeds had low levels of phthalic acid except at the 600 ppm loading rate where uptake increased substantially. Levels in mature wheat plants and seeds may be a result of the transplant of control plants into these pots which was necessary to replace dead plants. Uptake in mature wheat plants and seeds grown continuously in phthalic acid-treated soil would probably be higher. Mature soybean plants and seeds had relatively high levels of phthalic acid uptake. In all cases, uptake was considerably less than the oral LD_{50} toxicity dose for rats. For instance, to ingest 3.2 gms of phthalic acid (oral LD_{50} dose) from immature wheat plants at 600 ppm soil loading rate (the situation with the greatest uptake), a 1 kg rat would have to ingest 213 kg dry weight of immature wheat plants.

TABLE 13

AMOUNT AND STANDARD DEVIATION OF EXTRACTABLE PHTHALIC ACID REMAINING IN SOIL AFTER FINAL HARVEST (74 DAYS)

ppb-dry weight

Top Soil

Initially Applied ppm	Corn and Wheat pots	Soybean and Fescue pots
0	0	0
0.6	0.2(0.1)	0.5(0.1)
6.0	3.0(0.7)*	6.0(1.0)*
60.0	33.5(4.6)*	91.0(24.5)*
600.0	262.2(64.0)*	397.6(147.7)*
Statistics:		
F	16.22**	9.86**
L.S.D. (t at .05)	0.42	0.70

Bottom Soil

Initially Applied ppm	Corn and Wheat pots	Soybean and Fescue pots
0	0	0
0	0.1(0.1)	0.2(0.0)
0	1.8(1.2)*	1.3(0.5)*
0	9.9(0.6)*	4.9(2.3)*
0	58.8(43.0)*	185.8(169.9)*
Statistics:		
F	14.90**	58.32**
L.S.D. (t at .05)	0.23	0.11

* Statistically significant at $p \leq 0.05$.

** Statistically significant at $p \leq 0.01$.

Data log transformed after changing all data to X + 1, for statistical analysis

Phthalic acid was rapidly lost from the experimental pots. By the end of the experiment, only 5.7% of the originally applied ^{14}C-phthalic acid was present in soil or plants. Plants only contained 0.3% of the recovered ^{14}C. A small amount of phthalic acid leached into the bottom soil. Microbial decomposition is probably the main vector of phthalic acid loss from the soil rather than leaching, plant uptake or volatilization.

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SECTION 6

CROP UPTAKE AND SOIL RETENTION OF PHTHALIC ACID ESTERS: DI-*N*-BUTYL PHTHALATE (DnBP), DI-N-OCTYL PHTHALATE (DnOP) AND DI-ETHYL HEXYL PHTHALATE (DEHP)

MATERIALS AND METHODS

This study was the second greenhouse investigation and represents three members of the phthalic acid ester group of priority pollutants. The basis for loading rates and the general procedures used were presented in earlier sections of this report.

CHEMICAL

^{14}C labeled chemicals were added to unlabeled analytical grade chemicals at rates shown in Table 14 (DnBP - Fisher Reagent grade D30, DnOP-Eastman Lot 6429 and DEHP).

The procedures used to synthesize ^{14}C-labeled PAE's are those described by Albro, Thomas and Fishbein (1973) and Albro and Thomas (1973). Phthalic anhydride (4 mg, Matheson, Coleman and Bell, 99.5%), *n*-butyl alcohol (1 ml, Mallinkrodt analytical reagent, b. p. 117-118^{o}) and *p*-toluene- sulfonic acid (1 mg, Matheson, Coleman, and Bell, m.p. 104-106^{o}) in 2 ml of toluene were heated at 70^{o} overnight. The organic phase was washed twice with 0.4 M K_2CO_3, dried over molecular sieves and then concentrated at 55^{o}C (rotary evaporator) to about 0.5 ml.

Separation and final purification of the desired di-*n*-butyl phthalate acid ester were achieved by thin-layer chromatography (TLC) on Silica Gel FG (1000 thickness) using benzene-methyl alcohol-acetic acid (11:2:1) as developing solvent. The structure of the ester was confirmed by the method of preparation, by TLC comparison with an authentic sample and by spectroscopic analysis (infrared, NMR and mass spectroscopy).

Carbonyl^{14}-labeled di-*n*-butyl phthalate (DnBP) was synthesized as above by reacting 0.86 mg [7- ^{14}C) phthalate anhydride (Amersham Corporation, 0.33 mCi, 58 mCi/mmole) and 0.5 ml of *n*-butyl alcohol in the presence of 1 mg-toluenesulfonic acid. The final product, purified as described above, contained 82% of the starting ^{14}C from labeled phthalic anhydride and had a specific radioactivity of 209 uCi/mg (by liquid scintillation). Cold and carbonyl-^{14}C-labeled di-2-ethylhexyl phthalate (DEHP) and di-*n*-octyl phthalate (DnOP) were synthesized by appropriate modifications of the above procedure. Carbonyl-^{14}C-labeled di-2-ethylhexyl phthalate had a specific radioactivity of 148 uCi/mg and contained 67% of the starting ^{14}C from labeled phthalic anhydride. Carbonyl-^{14}C-labeled di-*n*-octyl phthalate had a specific radioactivity of 148 uCi/mg and contained 72% of the starting ^{14}C from labeled phthalic anhydride.

TABLE 14. AMOUNTS OF PHTHALIC ACID ESTERS APPLIED TO POTS IN GREENHOUSE STUDIES

Chemical	Applied Rates		
	1X	10X	100X
Di-*n*-butyl phthalate (DnBP)			
Total mg/pot	0.13	1.28	12.86
ppm	0.02	0.22	2.20
kg/ha	0.04	0.40	4.00
lb/acre	0.03	0.33	3.29
Di-*n*-octyl phthalate (DnOP)			
Total mg/pot	0.13	1.28	12.76
ppm	0.02	0.22	2.20
kg/ha	0.04	0.40	4.00
lb/acre	0.03	0.33	3.29
Di-ethyl hexyl phthalate (DEHP)			
Total mg/pot	2.55	25.52	255.20
ppm	0.44	4.40	44.00
kg/ha	0.80	7.90	78.70
lb/acre	0.70	7.05	70.20

CROP PLANTS

Problems were encountered in achieving germination and growth because the ethanol used as a solvent for the chemicals removed soluble salts from the Osmocote^R. Seeds were planted on 11/24/80, and replanted on 12/15/80 and 1/7/81. On 1/7/81, pots were heavily leached with water to remove the salts. On 2/4/81, the top four inches of soil were removed and replaced with soil containing the respective chemicals. Successful germination occurred from the final planting on 2/9/81.

Corn and fescue plants were harvested at 17 and 34 days after planting, respectively. Soybean and wheat plants were thinned and retained for analysis at 34 days and the remaining plants were harvested when mature at 93 and 96 days, respectively. Wheat and soybean seeds were separated from the nonedible plant portions by hand. Weeds were also harvested and retained for analysis when the final crop was sampled.

CHEMICAL ANALYSIS

Plant samples were homogenized for 2 to 3 minutes with a "45" Virtis homogenizer at 39,000 RPM. Twenty-five ml of hexane:acetone (1:1 - Fisher AH-300 pesticide grade and Fisher A-18 certified grade, respectively) were used for each sample. After homogenization, blades were washed into the grinding flask and flask contents were filtered through a Whatman GF/B 2.4 cm Glass Microfiber filter under suction into a side-armed Erlenmeyer flask. The flask was washed with hexane:acetone into a 250 ml beaker, placed under a hood and dried. Four ml of hexane were added to redissolve the filtrate, and the beaker was ultrasonicated. A two ml sample was removed and retained for later analysis. Several drops of sodium hypochlorite bleach (Chlorox^R) were added to the vials to bleach out the chlorophyll in the remaining 2 ml of filtrate.

Scintillation cocktail [16.5 gm 2.5-diphenyloxazole (PPO), 0.5 gm 1,4-bis[2-(4-methyl-5-phenyloxozyl)] benzene (POPOP); 1000 ml Triton X-100 and 200 ml toluene] was added to each of the vials. A liquid scintillation spectrometer (Packard Tri-Carb Model 2405) was used to twice assay each vial (20 minutes or 20,000 counts). Observed radioactivity was corrected for counting efficiency which ranged from 73 to 85%.

In chemical extraction from the soil, a 30 to 40 gm soil sample was weighed, placed in a beaker with 50 ml of hexane:acetone (1:1) and ground for two minutes with a tissue homogenizer (Tek-Mar Homogenizer). After settling for 30 minutes, the sample was filtered through Whatman Glass Microfiber GF/B filter paper into a side-armed Erlenmeyer flask. The filtrate was transferred to a 250 ml beaker and evaporated to dryness under a hood. It was transferred to a scintillation vial and evaporated with a Buchler Vortex-Evaporator ($T=80^oC$, P=20"Hg and Vortex = 5). Four ml of hexane were added to each vial, allowed to stand overnight and then ultrasonicated. Two ml were retained for later analysis and two ml were radioassayed using the same methods as described previously.

EXTRACTION EFFICIENCIES

A mixture of unlabeled and labeled C^{14}-labeled PAE were injected into mature corn and soybean stems with a microliter syringe. After 24 hours, the plants were harvested and analyzed to determine extraction efficiencies. For soil samples, a predetermined amount of labeled PAE was mixed into soil samples in an Erlenmeyer flask. After 2 hours, they were extracted and analyzed. For both plant and soil samples, C^{14}-chemical injection levels were similar to those found in extracted samples. Extraction efficiencies of ^{14}C were generally consistent and high. Plant extraction efficiencies were 96% for DEHP, 89.7% for DnBP and 100% for DnOP. Soil extraction efficiencies were 89.2% for DEHP, 88.65 for DnBP and 84.3% for DnOP.

CHEMICAL CONCENTRATION CALCULATIONS

Concentrations of the PAE's were calculated by subtracting background counts (from control plants); multiplying by two (to account for sample splitting) and divided by sample fresh weight to yield, C^{14} activity expressed as disintegrations per minute per gram of fresh weight (dpm/gfw). A conversion factor (mg labeled X dpm/mg)/(mg labeled + mg unlabeled x 10^3) was calculated. The inverse of this value when multiplied by sample dpm/gfw resulted in ppm of organic chemical in the sample. This value was divided by extraction efficiency and multiplied by percent dry weight and to yield parts per billion dry weight.

Total harvest fresh weights for each crop was multiplied by their respective dpm/gfw for each pot. These were summed to determine total aboveground plant radiation recovered. Bottom (5 cm) and top (12 cm) soil samples were treated in a similar fashion. To estimate root biomass, all roots were removed from 24 DnBP pots which grew wheat and corn. Soil was sieved by hand through two soil sieves (4.00 mm and 1.70 mm). Roots were weighed, a subsample dried and the remainder frozen for later chemical analysis. Root:shoot ratios were calculated and used to yield total root dpm. The total dpm found in aboveground plants, roots and soil were summed and extraction efficiencies included. Total dpm accounted for was divided by the number of dpm originally applied to calculate percent recovery.

STATISTICAL ANALYSIS

Data were analyzed with the General Linear Models (GLM) and MEANS procedures from SAS (Barr et al 1979). Plant uptake and soil retention data were $\log_{10}$ transformed after converting adding 1 to all data ($X + 1$) for the GLM procedure to calculate analyses of variance (Snedecor and Cochran 1967). For plant dry weight and height, variables used in analyses of variance were replicate, chemical, rate, and rate x chemical interaction. Plant uptake and soil retention were analyzed by chemical and plant. The variables were rate and replicate.

RESULTS AND DISCUSSION

Plant uptake and soil retention are discussed assuming that the C^{14} detected is the parent compound.

PLANT GROWTH

In general, there was no significant effect on plant height of chemical, rate, replicate or rate x chemical interaction (Table 15). Similarly, there was no significant effect on plant dry weight of chemical, rate, replicate or rate x chemical (Tables 16 and 17) except for soybean seeds which had lower dry weights with increasing rate for DnBP and DnOP.

PLANT UPTAKE

Plant uptake was low and ranged from zero to 4.6 ppm. It increased with increased loading rate (Table 18 and 19). Uptake also varied by plant. For all three chemicals, uptake was greatest in fescue and corn and least for soybean pods and immature soybean. Mature wheat plants and weeds from soybean-fescue pots had the second highest uptake rates. Uptake was greatest for DnOP which reflects its higher loading rate. Uptake was higher for DEHP than DnBP.

Uptake of DnBP was significantly different from zero for fescue, soybean seed ($p \leq .10$) and mature wheat plants. In these plants, as well as wheat seed and weeds from soybean - fescue pots, uptake was significant at the 100X rate. In addition, mature wheat straw and fescue uptake were significant at the 10X rate and fescue uptake was also significant at the 1X rate. Uptake was not significant at any rate for corn, immature soybean plants, soybean seed and pods, immature wheat plants and weeds from wheat pots. For DEHP, uptake was significantly different from zero ($p \leq .05$) for all crops except mature and immature soybean plants, and soybean seeds and pods. Uptake was significant for immature wheat at ($p \leq .10$). For these, uptake was significant at all rates except that uptake was significant only at the 10X and 100X rates for immature wheat plants and wheat seed. For DnOP, uptake was significantly different from zero only for corn, fescue, mature wheat plants, wheat seed and weeds from soybean pots. Uptake was significant at the 10X and 100X rates for corn and fescue. Uptake was significant at the 100X rates for mature wheat plants, wheat seeds and weeds from soybean pots. Uptake was not significant at any rate for mature and immature soybean plants and soybean pods.

SOIL RETENTION

Statistically significant amounts of chemicals remained in the soil for both sets of pots for all chemicals (Table 20). Soil concentrations were ranked DnBP<DnOP<DEHP. Amounts still present were significant at all rates except for the 1X rate for DnBP.

TABLE 15. HEIGHT (cm) AND STANDARD DEVIATIONS AT HARVEST OF PLANTS GROWN IN GREENHOUSE POTS TREATED WITH PHTHALIC ACID ESTERS

Chemical	Rate	Corn	Fescue	Immature Soybean	Mature Soybean	Immature Wheat	Mature Wheat
Control	0	21(6.1)	15(2.5)	19(1.7)	36(4.0)	20(8.4)	31(4.2)
Di-*n*-butyl phthalate	1X	28(2.3)	17(3.1)	22(1.0)	30(4.0)	26(3.2)	37(2.1)
	10X	26(8.5)	15(0.0)	28(5.5)	38(2.1)	26(6.9)	41(6.0)
	100X	29(3.1)	19(0.6)	24(3.2)	32(8.1)	28(3.2)	39(5.0)
Di-*n*-ethyl hexyl phthalate	1X	22(9.5)	15(3.1)	20(0.0)	32(4.7)	27(9.6)	36(4.0)
	10X	27(5.0)	17(3.5)	27(1.7)	35(4.0)	30(3.1)	40(1.5)
	100X	20(6.8)	13(2.5)	24(6.7)	31(5.6)	24(1.0)	36(6.5)
Di-*n*-octyl phthalate	1X	29(1.2)	13(1.5)	22(2.9)	29(9.0)	30(1.5)	35(1.0)
	10X	25(5.1)	14(4.4)	18(1.5)	29(8.1)	28(2.0)	38(2.1)
	100X	26(7.9)	15(2.1)	26(4.0)	33(6.1)	29(7.6)	40(12.3)
Statistics							
Average (S.D.)		25(5.8)	15(2.5)	23(3.6)	32(6.0)	27(5.5)	37(5.6)
L.S.D. (t=.05)		9.9	4.2	6.1	10.3	9.5	9.7
F		1.2	1.9	2.1	0.7	0.9	0.7

TABLE 16. DRY WEIGHT (gm) AND STANDARD DEVIATIONS OF PLANTS GROWN IN GREENHOUSE POTS TREATED WITH PHTHALIC ACID ESTERS

Chemical	Rate	Fescue	Immature Soybean	Mature Soybean	Soybean Seed	Soybean Pod
Control	0	1.8(0.4)	6.3 (0.0)	7.5(4.6)	5.0(0.8)	2.4(0.9)
Di-*n*-butyl phthalate	1X	2.6(1.0)	8.6 (4.2)	9.0(4.2)	5.0(0.0)	4.1(0.0)
	10X	2.3(0.5)	17.3(12.2)	10.9(4.7)	8.6(3.1)	4.4(0.4)
	100X	3.2(0.8)	4.8 (2.2)	6.8(2.4)	0.4(0.4)	1.0(0.6)
Di-*n*-ethyl hexyl phthalate	1X	2.9(1.2)	5.1 (2.0)	6.1(0.8)	0.4(0.0)	1.5(0.1)
	10X	1.8(0.1)	17.0 (7.4)	9.9(3.2)	1.1(0.8)	1.2(1.8)
	100X	1.3(0.5)	7.6 (8.2)	6.9(4.1)	2.8(3.4)	1.3(1.5)
Di-*n*-octyl phthalate	1X	1.4(0.2)	3.3 (0.1)	6.5(3.0)	3.1(0.0)	1.3(1.5)
	10X	2.3(1.4)	3.1 (2.4)	6.2(2.2)	- -	0.6(0.0)
	100X	1.9(0.2)	13.5 (6.4)	6.8(1.5)	0.7(0.6)	1.0(0.1)
Statistics		2.1(0.8)	9.1 (6.6)	7.6(2.9)	2.9(1.8)	1.8(1.1)
Average S.D.		1.4	11.8	5.0	3.5	2.0
L.S.D. (t=.05)		1.5	1.3	1.5	3.8	2.3
F						

TABLE 17. DRY WEIGHT (gm) AND STANDARD DEVIATIONS OF PLANTS GROWN IN GREENHOUSE POTS TREATED WITH PHTHALIC ACID ESTERS

Chemical	Rate	Corn	Immature Wheat	Mature Wheat	Wheat Seed	Weeds	Soybean Seed
Control	0	2.0(1.1)	4.8(0.0)	4.4(0.0)	4.0(0.0)	4.0(0.0)	5.2(2.3)
Di-*n*-butyl Phthalate	1X	2.4(0.9)	2.0(0.4)	5.1(2.3)	4.6(2.2)	4.6(2.8)	4.8(2.1)
	10X	2.5(1.0)	2.4(1.0)	3.8(1.3)	4.3(2.2)	6.2(1.2)	3.8(2.1)
	100X	2.8(0.5)	2.2(0.4)	3.3(0.6)	3.7(0.6)	5.6(2.5)	5.6(1.2)
Di-*n*-ethyl hexyl phthalate	1X	1.9(1.6)	2.1(0.1)	2.2(0.3)	3.2(1.4)	4.1(0.8)	6.3(1.0)
	10X	2.7(0.7)	2.8(0.4)	4.7(3.2)	6.1(1.8)	5.0(0.8)	4.6(2.2)
	100X	1.6(0.8)	1.9(1.4)	3.3(1.4)	2.6(0.2)	3.9(2.2)	3.7(2.1)
Di-*n*-octyl phthalate	1X	3.0(0.5)	2.6(1.0)	4.2(1.6)	5.2(0.9)	5.7(1.3)	2.5(1.0)
	10X	2.1(0.7)	1.8(0.3)	3.8(1.2)	3.8(2.6)	6.0(1.1)	7.0(0.7)
	100X	2.3(1.1)	1.1(1.0)	2.6(0.9)	4.4(1.4)	7.8(2.7)	4.2(1.3)
Statistics							
Average S.D.		2.3(1.0)	2.1(0.8)	3.7(1.6)	4.2(1.7)	5.4(1.9)	4.8(1.5)
L.S.D. (t=.05)		1.6	1.5	2.9	2.9	3.3	2.6
F		0.7	0.7	0.9	0.9	1.1	2.4

TABLE 18. UPTAKE OF PHTHALIC ACID ESTERS AS MEASURED 14C IN GREENHOUSE-GROWN PLANTS

Parts per billion[a]

Chemical	Rate	Fescue	Immature Soybean	Mature Soybean	Soybean Seed	Soybean Pod
Di-*n*-butyl phthalate	0	0.0	0.0	0.0	0.0	0.0
	1X	1.6*	0.0	0.0	0.0	0.0
	10X	7.9*	0.0	0.0	1.1	0.2
	100X	81.2*	0.0	4.4*	0.0	0.0
Statistics						
L.S.D.		1.4	-	4.1	1.4	1.3
F		201.9*	-	2.6	16.9**	20.0
Di-*n*-octyl phthalate	0	0.0	0.0	0.0	0.0	0.0
	1X	0.7	0.0	0.0	0.0	0.0
	10X	5.0*	0.0	0.0	-	0.0
	100X	44.3*	0.0	0.9	0.0	0.0
Statistics						
L.S.D.		2.7	-	2.1	-	-
F		20.7*	-	1.7	-	-
Di-ethyl hexyl phthalate	0	0.0	0.0	0.0	0.0	0.0
	1X	28.4*	0.0	0.0	0.0	0.0
	10X	266.7*	0.0	12.0	3.6	2.7
	100X	3191.1*	0.0	10.8	122.0*	0.0
Statistics						
L.S.D.		1.4	-	17.3	6.7	95808.3
F		393.8*	-	1.8	3.06	0.6

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* Significant at $P < .05$

** Significant at $P \leq .10$

[a] All data $\log_{10}$ transformed after adding X + 1 for statistical analyses

TABLE 19. UPTAKE OF PHTHALIC ACID ESTERS AS MEASURED 14C IN GREENHOUSE-GROWN PLANTS

Parts per billion[a]

						Weeds	
Chemical	Rate	Corn	Immature Wheat	Mature Wheat	Wheat Seed	Wheat Pot	Soybean Pot
Di-*n*-butyl phthalate	0	0.0	0.0	0.0	0.0	0.0	0.0
	1X	0.9	0.0	0.1	0.0	0.1	0.2
	10X	2.0	0.2	1.3*	0.1	0.6	2.6
	100X	97.3	0.0	13.2*	6.6*	3.6	11.0*
Statistics							
L.S.D.		62034.4	1.4	1.2	3.6	4.1	8.2
F		3.7	0.7	441.3*	5.0	1.8	1.9
Di-*n*-octyl phthalate	0	0.0	0.0	0.0	0.0	0.0	0.0
	1X	0.6	0.0	0.1	0.0	0.1	0.1
	10X	6.7*	0.0	1.0	0.2	0.4	1.2
	100X	35.9*	0.0	10.0*	3.1*	0.7	9.1*
Statistics							
L.S.D.		1.9	-	1.2	1.4	3.8	1.4
F		85.4*	-	562.9*	37.2*	.32	76.1*
Di-ethyl hexyl phthalate	0	0.0	0.0	0.0	0.0	0.0	0.0
	1X	8.7*	0.0	4.6*	0.5	3.6*	2.9**
	10X	21.9*	13.1*	30.0*	6.4*	1.9	30.8*
	100X	4568.2*	106.8*	314.6*	64.8*	17.4*	317.7*
Statistics							
L.S.D.		4.0	8.8	1.9	1.7	3.0	3.8
F		52.3*	158.2**	81.6*	91.8*	5.3*	21.7*

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* Significant at $P < .05$

** Significant at $P \leq .10$

[a] All data $\log_{10}$ transformed after adding X + 1 for statistical analyses.

TABLE 20. TOP SOIL RETENTION OF PHTHALIC ACID ESTERS AS MEASURED 14C

Parts Per Billion (dry weight)[a]

Chemical	Rate	Initially Applied	Wheat and Corn Pots	Soybean and Fescue Pots
Di-*n*-butyl phthalate	0	0	0	0
	1X	22.0	0.5	0.7
	10X	220.0	5.3*	4.6*
	100X	2200.0	21.9*	39.8*
Statistics				
L.S.D.			3.3	1.2
F			98.4*	437.0*
Di-*n*-octyl phthalate	0	0	0	0
	1X	22.0	3.4*	3.7*
	10X	220.0	32.8*	30.2*
	100X	2200.0	265.3*	257.8*
Statistics				
L.S.D.			1.5	1.3
F			280.2*	732.6*
Di-ethyl hexyl phthalate	0	0	0	0
	1X	44.0	95.7*	100.3*
	10X	440.0	893.1*	863.2*
	100X	4400.0	5385.0*	4830.0*
Statistics				
L.S.D.			1.6*	1.3*
F			416.2*	1486.3*

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* Significant at $P \leq .05$

[a] Data $\log_{10}$ transformed after adding X + 1 for statistical analyses.

Based on analysis of DPM balance sheets (Table 21), most of each chemical had volatilized or been decomposed by the end of the experiment. DEHP was the most resistant to loss (an average of 24.8% recovered) followed by DnOP (20.0%) and DnBP (3%). Of the total recovered DPM, very little was found in the aboveground plant tissue; 4.0% for DEHP, 4.9% for DnOP and 3.8% for DnBP.

CONCLUSIONS

Uptake rates reflected initial loading rates and water solubility of the chemical. DEHP was loaded at the highest rates and had the most uptake. DnBP is the most water soluble chemical and had higher uptake than DnOP. Uptake of DEHP and DnBP were similar when loading rates are taken into account. Fescue and corn had the greatest uptake while uptake from wheat and soybean was minimal. Soil decomposition was highest for DnBP and least for DEHP which agrees with Johnson and Lulves (1975) in freshwater hydrosil.

TABLE 21. TOTAL DPM RECOVERED AT END OF EXPERIMENT AND PERCENT OF TOTAL DPM RECOVERED IN ABOVEGROUND PLANT TISSUE

	Total Percent DPM's Recovered			Percent of Total DPM Recovered in Aboveground Plant Tissue		
Chemical	Corn and Wheat Pots	Soybean and Fescue Pots	Average	Corn and Wheat Pots	Soybean and Fescue Pots	Average
DnBP	2.8	3.3	3.0	6.6	1.1	3.8
DEHP	25.6	24.0	24.8	5.7	2.2	4.0
DnOP	20.2	19.8	20.0	5.9	3.8	4.9

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SECTION 7

CROP UPTAKE AND SOIL RETENTION OF PHTHALIC ACID ESTERS; DI-N-BUTYL PHTHALATE AND DI-ETHYL PHTHALATE

This study was the third greenhouse investigation and represents one chemical used in study two as well as two radioisomers of di-ethyl phthalate (ring labeled, DEP, and carbonyl labeled, CDEP). Subsequent studies also utilized di-n-butyl phthalate as a routine priority pollutant chemical.

MATERIALS AND METHODS

CHEMICAL

^{14}C labeled chemicals were added to cold chemicals at the rates shown on Table 22.

CROP PLANTS

Seeds were planted on July 31, 1981. Height measurements were made weekly. All corn plants were harvested at 30 days and all fescue at 51 days. Wheat was planted on September 3 to replace the corn and harvested 18 days later. Soybeans were thinned at 32 days and completely harvested when mature at 59 days. Mature wheat was harvested at 129 days. Wheat and soybean seeds were separated by hand from nonedible plant portions. Weeds were harvested and retained for analysis when the final crop was sampled.

CHEMICAL ANALYSIS

Plant samples were homogenized for 2 to 3 minutes with a "45" Virtis homogenizer at 39,000 RPM. Twenty-five ml of hexane:acetone (1:1 - Fisher AH-300 pesticide grade and Fisher A-18 certified grade, respectively) were used. After homogenization, the blades were washed into the grinding flask and flask contents were then filtered through a Whatman GF-A 2.4 cm Glass Microfiber Filter under suction into a side-armed Erlenmeyer flask. The flask was washed with hexane:acetone into a 250 ml beaker and the beaker was placed under a hood. When evaporated to about five ml, the filtrate was washed into a glass scintillation vial and dried. Four ml of hexane were added to redissolve the filtrate, capped and left overnight. Vials were then ultrasonicated for four minutes, two ml removed and saved. Several drops of sodium hypochlorite bleach (Chlorox^{R}) were added to remove chlorophyll in the remaining two ml of filtrate. Vials were placed under fluorescent lighting to enhance bleaching.

TABLE 22. AMOUNTS OF PHTHALATE ACID ESTERS APPLIED TO POTS IN GREENHOUSE STUDIES

Chemical	Rates		
	1X	10X	100X
Di-*n*-butyl phthalate (DnBP)			
Total mg/pot	0.13	1.28	12.76
ppm	0.02	0.22	2.20
kg/ha	0.04	0.37	3.68
lb/acre	0.03	0.33	3.29
Di-ethyl phthalate (DEP)			
Total mg/pot	0.13	1.28	12.76
ppm	0.02	0.22	2.20
kg/ha	0.04	0.37	3.68
lb/acre	0.03	0.33	3.29
Di-ethyl phthalate (CDEP)			
Total mg/pot	0.13	-	12.76
ppm	0.02	-	2.20
kg/ha	0.04	-	3.68
lb/acre	0.03	-	3.29

Scintillation cocktail [16.5 gn 2.5-diphenyloxazole (PPO), 0.5 gm 1,4-bis [2-(4-methyl-5-[phenyloxazoyl]) benzene (POPOP); 1000 ml Triton X-100 and 200 ml toluene] was added to the vials. A liquid scintillation spectrometer (Packard Tri-Carb model 2405) was used to twice assay each vial (20 minutes or 20,000 counts). Observed radioactivity was corrected for counting efficiency which ranged from 77 to 89%. In chemical extraction from the soil, a 30 gram soil sample was weighed, placed in a 600 ml plastic beaker with 50 ml of hexane:acetone(1:1) and ground with a Tekmar-Homogenizer for two minutes. After settling for an additional 30 minutes, the sample was filtered under suction through Whatman GF/B Glass Microfiber 5.5 cm filter paper into a side-armed Erlenmeyer flask. The filtrate was transferred to a 250 ml beaker and dried under a hood until about five ml remained. The filtrate was then transferred to a scintillation vial and four ml of hexanes were added to the vial, let sit overnight and ultrasonicated. Two ml were retained and two radioassayed using the same methods as described previously.

EXTRACTION EFFICIENCIES

Predetermined amounts of ^{14}C labeled PAE were injected (into mature corn and soybean stems) with a microliter syringe. After 24 hours, the plants were extracted and analyzed to determine extraction efficiencies. For soil samples, a labeled amount of labeled PAE was injected into soil samples in a Erlenmeyer flask. After two hours, they were extracted and analyzed. For both plant and soil, ^{14}C - chemical injection levels were similar to those found in extracted samples. Extraction efficiencies were generally consistent and high except for CDEP (Table 23).

CHEMICAL CONCENTRATION CALCULATIONS

Concentrations of the PAE's were calculated by subtracting background counts (from control plants); multiplying by two (to account for sample splitting) and divided by sample fresh weight to yield decay per minute per gram of fresh weight (dpm/gfw). A conversion factor (mg-labeled x dpm/mg)/[(mg-labeled + mg unlabeled) x 10^3] was calculated. The inverse of this value when multiplied by sample dpm/gfw resulted in ppm. This value was divided by the extraction efficiency and percent dry weight and multiplied by 1000 to yield parts per billion dry weight.

Total harvest fresh weights for each crop were multiplied by their respective dpm/gfw for each pot. These were summed to determine total above-ground plant radiation recovered. Bottom (5 cm) and top (12 cm) soil samples were treated in a similar fashion. To estimate root biomass, all roots were removed by hand from DnBP pots which grew wheat and corn. Roots were weighed, a subsample dried and the remainder frozen for later chemical analysis. Root:shoot ratios were calculated and used to estimate root biomass. This value was multiplied by root dpm/gfw to yield total root dpm. The total dpm found in above-ground plants, roots and soil were summed and extraction efficiencies included. Total dpm accounted for were divided by the number of dpm originally applied to calculate percent recovery.

TABLE 23. ^{14}C-PHTHALATE ACID ESTERS PERCENT EXTRACTION EFFICIENCIES

Chemical	Plant			Soil
	Soybean	Corn	Average	
DnBP	86.9	95.4	91.1	78.5
DEP	36.8	59.3	50.3	58.4
CDEP	28.2	22.7	25.6	61.4

STATISTICAL ANALYSES

Data were analyzed with the GLM and MEANS procedures from SAS as described in earlier sections of this report. Plant uptake and soil retention data were $\log_{10}$ transformed after converting all data to $X + 1$ for the GLM procedure to calculate analyses of variance. Variables used in the ANOVAS were replicate and rate. For immature wheat, only rate was used to retain sufficient degrees of freedom.

RESULTS

Plant uptake and soil retention are described assuming that ^{14}C detected is the parent compound. At present, insufficient data exist to determine what proportion of the total ^{14}C detected is the parent compound or metabolites.

PLANT GROWTH

In general, there was no significant effect on plant height of chemical, rate, replicate pot or rate x chemical interaction (Tables 24 and 25). The only exceptions were an effect of chemicals on mature soybeans (DEP increased height relative to the control) and rate x chemical interaction for immature soybeans (Tables 26 and 27). Dry weights also showed, in general, no significant effect by these variables. Exceptions were chemical effect in corn (DEP increased weight), mature soybeans (DnBP decreased weight) and soybean seed (CDEP increased weight). Replicate pots had a significant effect on mature soybeans and the rate x chemical interaction was significant for immature soybeans. In general, these chemicals at these rates had little effect on harvest height or dry weight of any plant.

PLANT UPTAKE

Plant uptake was low and ranged from zero to 360 ppb. It increased with increasing loading rate (Tables 28 and 29). Uptake also varied by plant. For DEP and CDEP, soybean seeds and mature soybean plants had the most uptake while fescue and corn had the least uptake. For DnBP, soybean seeds, and mature soybean plants still had the most uptake while wheat seed and corn had the least uptake. Uptake also varied by chemical and rate. Overall, DEP was taken up the most, followed by CDEP and then DnBP.

For DEP, uptake was significantly different from zero ($p \leq .05$) for all plants except immature wheat plants. In all plants, except corn, overall uptake was significant at the 0.22 and 2.2 ppm rates. Uptake was significant at all rates for mature soybean plants and soybean seeds. For CDEP, uptake was significant in corn, fescue, immature soybean and soybean seeds. Immature and mature wheat uptake were significant at $p \leq .10$. Uptake was significant ($p \leq .05$) only at the 2.2 ppm rate but was also significant at $p \leq .1$ for soybean seeds at the 0.02 rate.

TABLE 24. HEIGHT (cm) AND STANDARD DEVIATIONS OF PLANTS GROWN IN GREENHOUSE POTS TREATED WITH PHTHALIC ACID ESTERS

Chemical	Rate ppm	Corn	Immature Wheat	Mature Wheat	Wheat Seed
None	0	60(5.8)*	20(3.8)	53(1.5)	-
	0	54(1.2)	21(0.6)	58(6.7)	-
Di-*n*-butyl phthalate (DnBP)	0.02	56(1.7)	25(2.6)	46(5.2)	-
	0.22	58(2.6)	23(2.6)	46(1.7)	-
	2.2	57(1.0)	23(2.6)	52(9.8)	-
Di-ethyl phthalate (DEP)	0.02	61(3.2)	26(6.9)	52(6.7)	-
	0.22	64(3.6)	19(4.5)	47(5.5)	-
	2.2	57(2.3)	18(2.0)	53(11.3)	-
Di-ethyl phthalate (CDEP)	0.02	58(1.5)	23(2.3)	47(5.2)	-
	2.2	59(3.6)	24(5.5)	49(3.8)	-
Statistics					
Average + S.D.		58(3.2)	22(3.6)	50(6.0)	-
L.S.D. (t=.05)		5.4	6.2	10.3	
F		1.9	1.6	1.5	

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*Height in cm (standard deviation)

TABLE 25. HEIGHT (cm) AND STANDARD DEVIATIONS OF PLANTS
GROWN IN GREENHOUSE POTS TREATED WITH PHTHALIC ACID ESTERS

Chemical	Rate ppm	Fescue	Immature Soybeans	Mature Soybeans	Soybean Seed
None	0	17(3.5)*	36(1.2)	34(3.0)	-
	0	23(2.1)	35(2.0)	34(1.5)	-
Di-*n*-butyl phthalate (DnBP)	0.02	18(0.6)	38(0.0)	36(2.5)	-
	0.22	18(2.1)	35(0.6)	34(3.0)	-
	2.2	19(1.2)	35(0.0)	35(1.2)	-
Di-ethyl phthalate (DEP)	0.02	18(1.0)	36(3.1)	36(0.0)	-
	0.22	18(1.5)	39(1.0)	41(2.0)	-
	2.2	18(2.1)	35(2.6)	36(2.6)	-
Di-ethyl phthalate (CDEP)	0.02	19(1.5)	36(2.6)	36(2.3)	-
	2.2	19(0.0)	38(1.5)	36(3.0)	-
Statistics					
Average + S.D.		18(2.0)	36(1.8)	36(2.4)	-
L.S.D. (t=.05)		3.5	3.1	4.1	
F		0.6	1.9	1.9	-

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*Height, in cm (standard deviation)

TABLE 26. DRY WEIGHT(gm) AND STANDARD DEVIATIONS OF PLANTS GROWN IN GREENHOUSE POTS TREATED WITH PHTHALIC ACID ESTERS

Chemical	Rate ppm	Corn	Immature Wheat	Mature Wheat	Wheat Seed
None	0	7.7(0.7)*	0.8(0.2)	12.1(2.4)	10.5(2.5)
	0	7.2(1.4)	0.8(0.2)	15.9(2.2)	12.6(5.3)
Di-n-butyl	0.02	7.5(0.3)	1.3(0.4)	10.0(4.9)	8.8(3.9)
phthalate	0.22	7.5(0.1)	0.9(0.2)	13.8(2.0)	11.9(1.0)
(DnBP)	2.2	7.8(0.4)	1.0(0.4)	12.1(3.2)	10.0(3.0)
Di-ethyl	0.02	9.2(1.9)	1.0(0.9)	13.0(0.6)	10.6(1.1)
phthalate	0.22	11.9(3.8)	0.7(0.4)	9.7(1.3)	7.0(3.2)
(DEP)	2.2	7.5(1.4)	0.7(0.4)	11.9(5.2)	10.8(4.3)
Di-ethyl	0.02	7.6(0.7)	0.9(0.2)	9.1(0.3)	7.1(0.4)
phthalate	2.2	8.4(1.4)	1.0(0.4)	11.6(2.3)	9.6(1.4)
(CDEP)					
Statistics					
Average + S.D.		8.2(1.5)	0.9(0.4)	11.9(3.0)	9.9(3.0)
L.S.D. (t=.05)		2.5	0.7	5.1	5.2
F		2.9	0.6	1.2	1.1

*Dry weight in gm (standard deviation)

TABLE 27. DRY WEIGHT (gm) AND STANDARD DEVIATIONS OF PLANTS GROWN IN GREENHOUSE POTS TREATED WITH PHTHALIC ACID ESTERS

Chemical	Rate ppm	Fescue	Immature Soybeans	Mature Soybeans	Soybeen Seed
None	0	1.8(0.7)*	6.8(0.0)	3.2(0.8)	6.0(0.5)
	0	3.9(1.4)	5.9(1.4)	5.9(1.4)	6.4(1.2)
Di-*n*-butyl	0.02	2.5(0.8)	5.8(0.5)	2.0(0.1)	7.2(0.1)
phthalate	0.22	1.9(0.2)	6.3(0.2)	2.3(0.4)	7.2(0.9)
(DnBP)	2.2	3.3(0.9)	6.3(0.5)	2.8(0.2)	6.6(0.4)
Di-ethyl	0.02	2.2(0.5)	7.2(1.2)	3.2(1.7)	7.0(1.4)
phthalate	0.22	2.6(0.3)	7.5(0.7)	3.0(0.8)	6.7(0.7)
(DEP)	2.2	1.9(0.7)	5.5(1.0)	3.3(1.0)	6.6(0.7)
Di-ethyl	0.02	3.0(0.9)	5.7(0.4)	2.4(0.3)	7.3(0.2)
phthalate	2.2	2.6(0.3)	6.7(1.0)	2.4(0.2)	8.1(0.2)
(CDEP)					
Statistics					
Average + S.D.		2.5(0.7)	6.4(0.8)	2.9(0.9)	6.9(0.8)
L.S.D. (t=.05)		1.3	1.4	1.5	1.3
F		1.0	2.3	2.2	1.7

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*Dry weight in gm (standard deviation)

TABLE 28. UPTAKE OF PHTHALIC ACID ESTERS AS MEASURED ^{14}C IN GREENHOUSE-GROWN PLANTS

Parts per Billion[a]

Chemical	Rate ppm	Fescue	Immature Soybean	Mature Soybean	Soybean Seed
Di-n-butyl phthalate (DnBP)	0	0.0	0.0	0.0	0.0
	0.02	0.1	0.0	0.7	0.9
	0.22	0.7	0.4	1.8*	6.0*
	2.2	3.6*	3.8*	14.0*	205.6*
Statistics					
L.S.D.		1.8	1.2	1.5	2.5
F		14.4*	147.3*	61.6*	50.6*
Di-ethyl phthalate (DEP)	0	0.0	0.0	0.0	0.0
	0.02	0.2	0.3	2.5*	2.7*
	0.22	1.7*	2.5*	22.6*	14.5*
	2.2	10.2*	34.9*	222.0*	114.9*
Statistics					
L.S.D.		1.5	2.4	1.9	2.0
F		76.2*	26.0*	86.2*	50.7*
Di-ethyl phthalate (CDEP)	0	0.0	0.0	0.0	0.0
	0.02	0.1	0.2	1.2	2.1**
	2.2	8.4*	14.6*	109.7	114.3*
Statistics					
L.S.D.		2.2	1.5	542.1	2.6
F		43.8*	289.2*	16.2	87.1*

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* Significant at $p \leq .05$

** Significant at $p \leq .10$

[a] All data $\log_{10}$ transformed after adding X + 1 for statistical analyses.

TABLE 29. UPTAKE OF PHTHALIC ACID ESTERS AS MEASURED ^{14}C IN GREENHOUSE-GROWN PLANTS

Parts per Billion[a]

Chemical	Rate ppm	Corn	Immature Wheat	Mature Wheat	Wheat Seed
Di-*n*-butyl phthalate (DnBP)	0	0.0	0.0	0.0	0.0
	0.02	0.0	0.0	0.1	0.0
	0.22	0.2	0.2	3.4*	0.1
	2.2	0.9	4.8*	40.6*	0.8
Statistics					
L.S.D.		1.1	2.5	1.5	1.9
F		77.0*	77.8**	137.7*	1.5
Di-ethyl phthalate (DEP)	0	0.0	0.0	0.0	0.0
	0.02	0.1	1.0	1.1	0.3
	0.22	1.1	16.1	13.6*	3.2*
	2.2	15.5*	57.3	69.3*	36.0*
Statistics					
L.S.D.		1.2	4729.5	3.9	2.0
F		363.1*	4.15	14.3*	47.9*
Di-ethyl phthalate (CDEP)	0	0.0	0.0	0.0	0.0
	0.02	0.0	0.2	0.7	0.2
	2.2	2.4*	20.2*	28.8*	3.5
Statistics					
L.S.D.		1.3	9.3	7.1	12.0
F		164.3*	18.33**	12.5**	2.0

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* Significant at $p \leq .05$

** Significant at $p \leq .10$

[a] All data $\log_{10}$ transformed after adding X + 1 for statistical analyses.

DnBP uptake was significant ($p \leq .05$) in all plants except wheat seed. again, uptake was only significant at the 2.2 ppm rate in all plants except corn and wheat seed. In addition, mature soybean plant, soybean seeds and mature wheat plant uptake was significant at the 0.22 ppm rate.

SOIL RETENTION

Statistically significant amounts of chemicals remained in the top soil for both sets of pots for all chemicals (Table 30). Soil concentrations at each loading rate was ranked DEP>DNBP>CDEP. Amounts present were significant for all pots at 2.2 ppm rates. Retention at the 0.2 ppm rate was significant for DnBP and DEP fescue and soybean pots and DEP in corn and wheat pots.

Most of the chemicals had volatilized or decomposed by the end of the experiment, based on analysis of dpm balance sheet (Table 31). Di-n-butyl phthalate was the most resistant to loss (9.6% left) followed by DEP (8.1%) and CDEP (1.0%). Most of the DPM recovered were in the soil. Of the total DPM recovered in plant tissue, only 1.1%, 2.8% and 6.3% were found as parent DnBP, DEP and CDEP respectively.

Very little soil leaching was evident. Data from the soybean and fescue pots were not used because samples were contaminated. For corn and wheat pots, less than 0.5% of the initially applied DPM were recovered in the bottom of the pots (Table 32). DEP leached the most (0.04%), followed by DNBP (0.02%) and CDEP (0.002%). Leaching was significant only for DEP at the 100X rate.

CONCLUSIONS

In general, uptake reflected chemical water solubility since the more water soluble chemical (di-ethyl phthalate) had more uptake than the less soluble di-n-butyl phthalate. Plant uptake was low. Mature soybean plants and soybean seeds had the most uptake for all three chemicals. Generally, uptake was significant only at the 2.2 ppm rate except mature soybean plant and seed which were significant also at the 0.2 rate. Fescue, immature soybean plants and mature wheat plants had significant uptake at the 0.22 rate for DEP. Soil retention indicates that microbial decomposition of di-ethyl phthalate proceeds by side chain splitting since final CDEP soil concentrations were lower than DEP. Soil concentrations of DnBP were higher at harvest than DEP which indicates that decomposition of DEP is more rapid than DnBP. Leaching rates followed water solubility with significant leaching occuring only for DEP (CDEP was probably too rapidly decomposed to leach significantly).

TABLE 30. SOIL RETENTION OF PHTHALIC ACID ESTERS AS MEASURED ^{14}C

Parts Per Billion[a]

		Top Soil		Bottom Soil	
Chemical	Rate ppm	Corn and Wheat Pots	Fescue and Soybean Pots	Initially Applied	Corn and Wheat Pots
Di-n-butyl phthalate (DnBP)	0	0	0	0	0
	0.02	1.2	2.1	0	0.0
	0.2	9.8	16.8*	0	0.1
	2.2	143.6*	153.7*	0	1.1
Statistics					
L.S.D.		1.4	1.2		2.0
F		314.1*	1025.8*		2.2
Di-ethyl phthalate (DEP)	0	0	0	0	0
	0.02	1.3	1.6	0	0.0
	0.2	17.3*	20.0*	0	0.5
	2.2	165.1*	186.3*	0	4.5*
Statistics					
L.S.D.		1.8	1.5		1.2
F		138.4*	185.5*		212.7*
Di-ethyl phthalate (CDEP)	0.02	0.1	0.3	0	0.0
	2.2	16.7*	25.6*	0	0.1
Statistics					
L.S.D.		1.5	1.3		1.5
F		316.8*	843.3*		1.2

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* Significant at $p \leq .05$

[a] All data $\log_{10}$ transformed after adding X + 1 for statistical analyses.

TABLE 31. TOTAL ^{14}C-DPM RECOVERED AT THE END OF THE EXPERIMENT AND PERCENT OF TOTAL ^{14}C-DPM RECOVERED IN ABOVEGROUND PLANT TISSUE

	Total Percent ^{14}C-DPM Recovered			Percent of Total ^{14}C-DPM Recovered in Aboveground Plant Tissue		
	Corn and Wheat Pots	Soybean and Fescue Pots	Average	Corn and Wheat Pots	Soybean and Fescue Pots	Average
Di-*n*-butyl phthalate	7.3	12.0	9.6	1.1	1.1	1.1
Di-ethyl phthalate	7.3	8.9	8.1	2.8	2.7	2.8
Di-ethyl phthalate (CDEP)	0.7	1.4	1.0	6.4	6.3	6.3

TABLE 32. PERCENT SOIL LEACHING OF ^{14}C-DPM

	Corn and Wheat Pots	Soybean and Fescue Pots	Average
Di-*n*-butyl phthalate (DnBP)	0.019	1.19	0.60
Di-ethyl phthalate (DEP)	0.042	0.74	0.39
Di-ethyl phthalate (CDEP)	0.002	0.164	0.08

SECTION 8

CROP PLANT GROWTH AND UPTAKE OF POLYNUCLEAR AROMATICS: ANTHRACENE, BENZ(A)ANTHRACENE, NAPHTHALENE, AND PHENANTHRENE

This study was the fourth greenhouse investigation and represents a second major group of organic priority pollutants as found in municipal sludge. The methods are described in detail as an earlier part of this report with modifications for PNA's given in this section.

INTRODUCTION

The four polynuclear aromatics (PNA's) examined in this research phase are composed of condensed benzene rings. Naphthalene (NT) ($C_{10}H_8$) is the simplest and consists of two, fused benzene rings. Of the four PNA's, it has the smallest molecular weight (128.19), lowest boiling point (218^{o}C), highest vapor pressure (4.9 x 10^{-2} torr) and highest water solubility (31.7 to 34.4 mg/liter). Anthracene (AT) and phenanthrene (PA) ($C_{14}H_{10}$) are composed of three fused benzene rings - AT is a linear chain while PA is not. Molecular weight (178.24) and boiling point (340^{o}C) are the same but AT has a lower vapor pressure (1.95 x 10^{-4} torr vs. 6.8 x 10^{-4} torr) and is less water soluble (0.045 to 0.073 mg/liter vs. 1.00 to 1.29 mg/liter) than PA. Benz(a)anthracene (BA) consists of four benzene rings fused in a non-linear pattern. Of the four PNA's, BA has the highest molecular weight (228.3), highest boiling point (435^{o}C), lowest vapor pressure (5 x 10^{-9} torr) and lowest water solubility (0.014 to 0.009 mg/liter) [Environmental Protection Agency, 1979].

MATERIALS AND METHODS

CHEMICALS

^{14}C-labeled chemicals were added to unlabeled analytical grade chemicals: 0.1, 1.0, and 10.0 ppm (0.16, 1.6, 16 kg/ha, respectively). Anthracene (Fisher O-1256, purified grade) was mixed with ^{14}C-anthracene (Amersham Code CFA 337, Batch 13, specific activity 1 mCi/mmol). Benz(a)anthracene (Kodak 4672) was added to ^{14}C-benz(a)anthracene (Amersham Code CFA 158, Batch 5; specific activity 49 mCi/mmol,211 uCi/mg; analyzed January 28, 1980). Naphthalene (Fisher N7 purified grade) was added to ^{14}C-naphthalene (Amersham Code CFA .36, Batch 5; specific activity 5 mCi/mmol, 39.1 uCi/mg; analyzed May 9, 1980). Phenanthrene (Kodak 599) was added to ^{14}C-phenanthrene (Amersham CFQ 2492; specific activity 19.3 mCi/mmol, 108 uCi/mg; analyzed July 7, 1978, and reanalyzed March 3, 1980). Osmocote^{R} 14-14-14 time-release fertilizer was added to each pot at a rate of 30 gm/pot (14.8 cc/pot) once every three months. Diazinon [(O,O-Diethyl O-)2-isopropyl-4 methyl-6-pyrimidinyl phosphorothioate)] was used as needed to control insect pests.

The mixtures of ^{14}C-labeled and analytical grade chemicals were thoroughly mixed into the upper 12 cm (5.8 kg) of the soil in each pot. A 1-ml sample of each chemical mixture was retained and analyzed to confirm loading rates. Three replicates of a randomized, complete block design were used with two crop combinations. Sixteen control pots were also included. A total of 22 pots were used per chemical.

CROP PLANTS

Four days after chemical loading, three pots from each treatment were planted with 30 wheat (Triticum aestivum L.) 'Butte' and 10 corn (Zea mays L.) 'Pioneer 3368A' seeds. Three additional pots were planted with 20 soybean [Glycine max (L.) Merr.] 'Altoona' and 2.5 cc of fescue (Festuca arundinacea Schreb.) 'Kentucky 31' seeds. All seeds were planted on August 17, 1981. Height measurements were made and recorded weekly. Corn and fescue plants were harvested at 30 and 45 days after planting, respectively. Additional wheat seeds were planted on September 18 to replace the corn and harvested as immature wheat 26 days later. Immature soybean plants were thinned and retained for analysis at 31 days and the remaining plants were harvested when mature at 63 days. Mature wheat was harvested at 105 days. Wheat and soybean seeds were separated from the nonedible plant portions by hand. Weeds were also harvested and retained for analysis when the final crop was sampled.

CHEMICAL ANALYSIS

Plant samples were homogenized for 2 to 3 minutes with a "45" Virtis homogenizer at 39,000 RPM. Twenty-five ml of hexane:acetone (1:1 - Fisher AH-300 pesticide grade and Fisher A-18 certified grade, respectively) were used for each sample. After homogenization, the blades were washed into the grinding flask and flask contents were then filtered through a Whatman GF/B 2.4 cm glass Microfiber Filter under suction into a side-armed Erlenmeyer flask. The flask was washed with hexane:acetone into a 250 ml beaker and the beaker placed under a hood. When evaporated to about 5 ml, the filtrate was washed into a glass scintillation vial and dried to about 2 ml. the vial was transferred to a Vortex-Evaporator (Buchler 3-2200) for about 20 minutes until dry ($T=40^{o}C$, P=25" + Hg, Vortes=3). Four ml of hexanes were added to redissolve the filtrate, and the vessel capped and left overnight. Vials were then ultrasonicated for 4 minutes, and 2 ml were removed and retained for later analysis. Several drops of sodium hypochlorite bleach (Chlorox^{R}) were added to the vials to bleach out the chlorophyll in the remaining 2 ml of filtrate.

Scintillation cocktail [16.5 gm 2.5-diphenyloxazole (PPO), 0.5 gm 1,4-bis [2-(4-methyl-5-phenyloxozoyl)] benzene (POPOP); 1000 ml Triton X-100 and 200 ml toluene] was added to each of the vials. A liquid scintillation spectrometer (Packard Tri-Carb Model 2405) was used to twice assay each vial (20 minutes or 20,000 counts). Observed radioactivity was corrected for counting efficiency which ranged from 73 to 85%.

In chemical extraction from the soil, a 30 to 40 gram soil sample was weighed, placed in a Erlenmeyer flask with 50 ml of hexane:acetone (1:1) and shaken for 30 minutes. After settling for an additional 30 minutes, the sample was filtered under suction through Whatman GF/B Glass Microfiber filter paper into a side-armed Erlenmeyer flask. The filtrate was transferred to a 250 ml round bottomed flask and flash evaporated in a rotary water bath at 34°C until dry (10 minutes). The flask was washed with hexane-acetone (1:1) into a scintillation vial. Hexane and acetone were then evaporated from the vial in a Vortex-Evaporator. Four ml of hexane was added to each vial, allowed to stand overnight and then ultrasonicated. Two ml were retained for later analysis and two ml were radioassayed using the same methods as described previously.

EXTRACTION EFFICIENCIES

Predetermined mixtures of unlabeled and ^{14}C-labeled PNA were injected into corn and soybean stems with a microliter syringe. After 24 hours, the plants were harvested and analyzed to determine chemical extraction efficiencies. For soil samples, a predetermined amount ofunlabeled and ^{14}C-labeled PNA was sprayed onto soil samples in an Erlenmeyer flask. After 2 hours, they were extracted and analyzed. For both plant and soil samples, ^{14}C-chemical injection levels were similar to those found in extracted samples. Chemical extraction efficiencies were generally consistent and high except for naphthalene in soil (Table 33).

TABLE 33. ^{14}C-POLYNUCLEAR AROMATIC CHEMICAL EXTRACTION EFFICIENCIES

	Plants			
Chemical	Soybean	Corn	Average	Soil
Anthracene	58.8	80.9	69.7	82.3
Benz(a)anthracene	89.8	99.4	93.5	86.7
Naphthalene	68.7	69.0	64.8	38.3
Phenanthrene	64.6	98.4	81.5	83.3

BENZ(A)ANTHRACENE DISAPPEARANCE STUDIES

Twelve pots of benz(a)anthracene were loaded at 1.0 ppm on July 15, 1982, to 5.8 kg of soil to check BA disappearance rates from soil. Soils were sampled at 0 and 1 hours, and at 1, 2, 5, 16, 30, 46, and 65 days after treatment and analyzed as described previously and below.

^{14}C EXTRACTION EFFICIENCIES

^{14}C extraction efficiencies were determined by oxidizing previously extracted plant and soil samples in a Harvey Biological Oxidizer OX-300. Total oxidizable DPM's per sample were added to total extractable DPM's to yield total DPM per sample. Extractable DPM divided by total DPM yielded percent ^{14}C extraction efficiency. Plants from pots loaded at 1.0 ppm were used to determine ^{14}C extraction efficiencies.

THIN LAYER CHROMATOGRAPHY

To determine the percentage of extractable ^{14}C which was the parent compound, a 2 ml aliquot of duplicate extractions was streaked onto 1000 um silica gel plate (Type GF, Sigma Chemical Company). Plates were developed in hexane:isopropyl ether (99:1). Parent compound rf values were determined using standards mixed with control sample extracts and exposed in a spark chamber (Birchover Inst. Co., 9.84% methane in argon). Plates were sectioned, scraped and counted. We determined the percent of the total ^{14}C on the plate which was located at the PNA rf. This percentage was multiplied by the ^{14}C ppb to yield ppb of the polynuclear aromatic. Where insufficient saved samples existed to conduct TLC analysis, averages of similar aged plants within a chemical were used. This was the case for immature wheat for all chemicals, anthracene soybean seed and naphthalene wheat seed.

GAS CHROMATOGRAPHIC ANALYSIS

Soil extracts were analyzed on a Varian 3700 with a 6 foot by 4 mm id glass column packed with GP 10% SP-2100 on 100/120 Supelcoport. Anthracene and benz(a)anthracene were analyzed on a ^{63}Ni constant-current ECD with oxygen added (1.5 ml/min) at the detector following the methodology of Miller et al. (1981). Operating conditions were T-detector = 300°C, T-injector = 250°C and N_2 carrier gas - 36 ml/min. Oven temperatures and retention times were 200°C and 8.4 minutes for AT and 250°C and 11.5 minutes for BA. Minimum levels of detection were 0.5 ng/ul for AT and 0.26 ng/ul for BA. Phenanthrene (PA) and naphthalene (NT) did not respond well to the ECD; they were analyzed by FID (H_2 = 60 ml/min, Air = 300 ml/min, H_2 = 36 ml/min; T-detector = 300°C and T-injector = 230°C). Oven temperature and retention time for NT was 180°C and 2.5 minutes. PA samples were analyzed at 180WoC for 5 minutes and then programmed at 25°C/min to 230°C for 3 minutes. Retention time for PA was 9.0 minutes. Minimum levels of detection were 5 ng/ul for NT and 1 ng/ul for PA.

AT and BA soil samples were cleaned with a Florisil^R column in a disposable pipette with 40 mm of deactivated Florisil^R (400°C for 24 hours; 6% water) with 10 mm of Na_2SO_4 on top. One ml of sample in hexanes was added to the column and AT or BA eluted with 2 ml of methylene chloride. Elutions were dried under N_2 (about 100 ml/min) in a scintillation vial topped with a modified Snyder column. One ml of hexane was then added to the vial for GC analysis. Extraction efficiencies were 56% for AT and 95% for BA. Soil samples for PA did not need cleanup before FID analysis. NT soil samples had ^{14}C levels below the minimum level of detection (870 ppb) and were not analyzed on the GC. The other PNA's had ^{14}C levels above the minimum level of detection for the 10 ppm loading rate. All plant samples were below the minimum level of detection making GC analysis impossible.

CHEMICAL CONCENTRATIONS CALCULATIONS

Concentrations of the ^{14}C-PNA's were calculated by subtracting background counts (from control plants); multiplying by two (to account for sample splitting) and dividing by sample fresh weight to yield disintegrations per minute per gram of fresh weight (dpm/gfw). A conversion factor ([mg labeled x dpm/mg]) : ([mg labeled + mg unlabeled] x 10^{-3}) was calculated. The inverse of this value when multiplied by ^{14}C-sample dpm/gfw and percent of ^{14}C as parent compound (from TLC) resulted in ppm of organic chemical in the sample. This value was divided by extraction efficiency, multiplied by percent dry weight and 1000 to yield parts per billion dry weight.

Total harvest fresh weights for each crop were multiplied by their respective dpm/gfw for each pot. These were summed to determine total aboveground plant radiation recovered. Bottom (5 cm) and top (12 cm) soil samples were treated in a similar fashion. To estimate root biomass, all roots were removed from the 22 benz(a)anthracene pots which grew wheat and corn. Soil was sieved by hand through two soil sieves (4.0 mm and 1.7 mm). Roots were weighed, a sub-sample dried and the remainder frozen for later chemical analysis. Root:shoot ratios were calculated and used to estimate root biomass. This value was multiplied by rood dpm/gfw to yield total root dpm. Total dpm's found in aboveground plants, roots and soil were summed and ^{14}C and chemical extraction efficiencies included. Where ^{14}C extractions were not available, averages of similar aged plants were used. Total dpm's accounted for were divided by the number of dpm's originally applied to calculate percent recovery. Therefore, ^{14}C balance accounts for all radiation in the pots throughout the entire experiment.

STATISTICAL ANALYSIS

Data were analyzed with the Means and General Linear Models (GLM) programs from SAS (Barr et al. 1979). Plant uptake and soil retention data were $\log_{10}$ transformed after converting all data to X + 1 for GLM procedure to calculate analysis of variance (Snedecor and Cochran 1967). For plant dry weight and height, variables used in analyses of variance were replicate, chemical, rate, and rate X chemical interactions. Plant uptake and soil retention were analyzed by chemical and variables used were rate and replicate.

RESULTS

PLANT GROWTH

There were no significant effects of chemical, rate or replication on total dry weight or height, at harvest, for any crop plant (Tables 34 and 35).

PLANT UPTAKE

Plant ^{14}C extraction efficiencies varied considerably (Table 36). They were generally lowest for wheat and soybean seed and highest for corn and fescue. ^{14}C benz(a)anthracene extraction efficiencies were generally higher than the other chemicals.

Average chemical uptake levels ranged from zero to 587 ppb for all plants and varied directly with loading rate (Table 37). Overall, immature wheat, corn and mature soybeans had the greatest uptake while soybean seed, mature wheat plants and wheat seed had the least. Uptake also varied by chemical and rate applied. For all plants, at all rates, uptake was ranked AT > BA > NT > PA.

For anthracene, uptake was statistically greater than zero ($p \leq 0.05$) for all plants at the 10.0 ppm rate and for all plants except mature wheat and wheat seed at the 1.0 ppm rate. Only corn uptake was significant at the 0.1 soil loading rate. Benz(a)anthracene uptake was significant for all plants at the 10 ppm rate except mature soybean plants and wheat seed. In addition, uptake was significant at the 1.0 ppm rate for corn and immature wheat plants. Naphthalene uptake was significant at the 10.0 ppm rate for all plants except mature wheat straw. Uptake at the 1.0 rate was significant for fescue, immature soybean plants, soybean seed, and immature wheat plants. Phenanthrene uptake was significant only at the 10.0 ppm rate for all plants except fescue. Uptake at the 1.0 ppm rate was significant only for immature wheat plants.

Regression equations were produced to predict plant uptake from soil loading rate (Table 38). Statistically significant equations ($p \leq 0.05$) were generated for most plants and chemicals. Exceptions were benz(a)anthracene for immature and mature soybean plants and wheat seed, mature wheat plants for naphthalene and fescue for phenanthrene. Significant equations were also produced for immature plants (corn, fescue, soybeans and wheat), mature plants (wheat and soybean) and seed (wheat and soybean). these equations were generally less accurate than equations for a single species. These regression equations can be used to predict plant uptake (ppb) of these polynuclear aromatics based on soil loading rates (ppm).

TABLE 34. HEIGHT (cm) AND STANDARD DEVIATIONS OF PLANTS GROWN IN GREENHOUSE POTS TREATED WITH POLYNUCLEAR AROMATICS

Chemical	Rate (mg/kg)	Corn		Fescue		Immature Soybeans		Mature Soybeans		Immature Wheat		Mature Wheat	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Anthracene	0	62	(3.5)	20	(0.0)	34	(0.0)	40	(2.8)	21	(1.4)	52	(5.0)
	0.1	64	(0.6	21	(2.5)	38	(1.0)	44	(1.7)	25	(2.7)	46	(3.8)
	1.0	63	(5.7)	20	(1.2)	36	(2.7)	41	(5.7)	24	(4.0)	44	(0.6)
	10.0	65	(2.7)	20	(2.0)	34	(2.9)	39	(2.5)	24	(5.5)	45	(2.7)
Benz(a)-anthracene	0	60	(1.4)	16	(0.7)	34	(2.1)	38	(0.7)	23	(1.4)	44	(7.8)
	0.1	62	(5.9)	20	(1.0	36	(1.0)	40	(2.9)	22	(2.9)	44	(1.0)
	1.0	58	(2.7)	21	(2.3)	38	(1.5)	42	(4.7)	25	(3.5)	43	(2.0)
	10.0	63	(1.5)	21	(2.5)	36	(1.5)	38	(3.5)	27	(3.1)	48	(5.9)
Naphthalene	0	61	(1.4)	16	(1.4)	36	(2.8)	40	(3.5)	27	(2.8)	50	(6.4)
	0.1	62	(7.6)	19	(2.7)	40	(3.5)	41	(4.6)	22	(4.0)	43	(5.2)
	1.0	63	(1.7)	16	(1.0)	36	(2.7)	40	(4.0)	27	(4.0)	45	(6.5)
	10.0	64	(2.7)	20	(3.2)	34	(2.5)	39	(5.0)	22	(1.7)	53	(7.1)
Phenanthrene	0	64	(2.1)	18	(6.4)	35	(0.7)	38	(4.2)	27	(1.4)	43	(1.4)
	0.1	59	(4.0)	18	(1.7)	36	(1.7)	39	(3.6)	26	(3.1)	53	(4.6)
	1.0	62	(8.9)	17	(3.2)	36	(2.1)	41	(2.1)	25	(3.6)	47	(6.7)
	10.0	63	(2.0)	15	(4.2)	37	(1.0)	40	(4.0)	22	(1.2)	48	(2.5)
Average and S.D.		62	(4.3)	19	(2.6)	36	(2.1)	40	(3.8)	24	(3.3)	47	(4.6)
L.S.D.*		7.4		4.5		3.7		6.6		5.4		7.4	

*Least significant difference at $p \leq 0.05$.

TABLE 35. DRY WEIGHT (GM) AND STANDARD DEVIATIONS OF PLANTS GROWN IN GREENHOUSE POTS TREATED WITH POLYNUCLEAR AROMATICS.

Chemical	Rate (mg/kg)	Corn		Fescue		Immature Soybeans		Mature Soybeans		Soybean Seed		Immature Wheat		Mature Wheat		Wheat Seed	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Anthracene	0	10.0	(0.6)	3.0	(0.3)	5.1	(0.6)	8.0	(0.5)	7.0	(0.2)	0.8	(0.2)	10.1	(0.6)	9.6	(1.7)
	0.1	10.4	(0.8)	3.3	(0.6)	6.8	(1.5)	8.2	(1.1)	7.9	(0.5)	1.2	(0.4)	8.4	(1.9)	7.1	(0.8)
	1.0	10.9	(1.8)	3.0	(0.2)	6.0	(0.2)	9.1	(0.3)	8.2	(2.0)	1.2	(0.4)	8.7	(2.3)	8.3	(2.3)
	10.0	11.1	(1.3)	2.8	(0.4)	5.6	(1.6)	7.9	(0.8)	8.2	(1.3)	1.1	(0.6)	7.9	(2.4)	6.6	(2.1)
Benz(a)-anthracene	0	7.7	(1.2)	2.1	(0.3)	5.2	(0.9)	7.6	(0.6)	7.4	(1.0)	1.0	(0.3)	10.6	(0.9)	8.6	(0.2)
	0.1	9.2	(1.7)	3.0	(0.6)	5.7	(0.6)	7.2	(1.4)	7.3	(0.7)	1.0	(0.3)	8.0	(0.6)	5.7	(1.4)
	1.0	8.4	(0.4)	3.1	(0.7)	5.8	(1.1)	8.7	(1.0)	8.5	(1.6)	1.2	(0.5)	7.3	(1.7)	5.6	(2.4)
	10.0	10.0	(1.5)	3.2	(0.3)	4.8	(0.9)	7.7	(0.6)	8.3	(0.9)	1.5	(0.2)	9.6	(1.1)	7.2	(3.0)
Naphthalene	0	9.0	(0.4)	2.6	(0.2)	4.6	(1.0)	7.7	(1.7)	8.2	(0.7)	1.7	(0.0)	11.2	(1.4)	8.5	(5.0)
	0.1	9.0	(1.9)	3.3	(0.8)	5.5	(0.4)	6.9	(0.7)	8.2	(1.2)	1.1	(0.5)	10.1	(3.0)	6.4	(4.3)
	1.0	10.3	(1.4)	2.7	(1.5)	5.1	(0.9)	7.7	(1.9)	8.5	(1.4)	1.0	(0.6)	8.2	(2.4)	6.6	(2.4)
	10.0	11.6	(0.4)	4.2	(1.5)	4.6	(0.8)	8.3	(1.2)	8.1	(1.2)	1.6	(0.2)	9.9	(1.7	7.4	(0.5)
Phenanthrene	0	10.5	(0.8)	3.4	(1.4)	4.8	(0.8)	8.2	(1.8)	7.6	(1.5)	1.8	(0.6)	11.7	(2.1)	7.9	(1.3)
	0.1	8.5	(0.8)	3.0	(0.3)	5.4	(1.0)	8.7	(1.6)	8.1	(1.1)	1.3	(0.4)	12.6	(1.5)	8.6	(1.7)
	1.0	9.3	(2.3)	2.7	(0.9)	5.5	(0.4)	7.6	(1.2)	8.1	(1.8)	1.4	(0.2)	12.0	(4.9)	8.3	(2.1)
	10.0	9.7	(1.1)	2.5	(1.7)	6.2	(0.6)	7.2	(0.7)	8.4	(1.2)	1.2	(0.1)	12.6	(2.4)	7.6	(1.3)
Average and S.D. (all pots)		9.7	(1.4)	3.0	(0.8)	5.5	(0.9)	7.9	(1.2)	8.0	(1.3)	1.3	(0.4)	9.9	(2.3)	7.4	(2.3)
L.S.D.*		2.4		1.3		1.4		2.0		2.1		0.7		3.9		4.0	

*Least significant difference at P ≤ 0.05

TABLE 36. PERCENT ^{14}C EXTRACTION EFFICIENCY FROM PLANTS AND SOIL IN GREENHOUSE GROWN POTS.

	Chemical			
Crop or Soil	Anthracene	Benz(a)-anthracene	Naphthalene	Phenanthrene
Corn	33.3	76.9	65.1	33.0
Fescue	52.7	30.0	30.5	23.3
Immature soybeans	14.4	34.8	59.5	25.4
Mature soybeans	9.7	65.2	51.2	16.8
Soybean seed	n.d.*	36.6	38.0	9.7
Mature wheat	44.2	10.3	95.7	13.7
Wheat seed	3.0	7.0	n.d.*	4.3
Soil	37.1	42.2	11.9	19.8

* n.d. = no data.

TABLE 37. UPTAKE OF POLYNUCLEAR AROMATICS IN GREENHOUSE GROWN PLANTS--PARTS PER BILLION DRY WEIGHT

Chemical	Rate (mg/kg)	Corn	Fescue	Immature soybean plants	Mature soybean plants	Soybean seed	Immature wheat plants	Mature wheat plants	Wheat seed
Naphthalene	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.1	0.0	0.4	0.5	0.1	1.7	0.4	0.6	0.0
	1.0	2.5	5.2*	9.4*	1.1	11.0*	6.0*	6.3	0.5
	10.0	86.2*	47.3*	32.0*	4.2*	59.3*	18.3*	0.0	29.7*
Statistics L.S.D.[a,b]		3.4	1.8	1.7	2.5	2.8	1.9	11.0	2.0
Phenanthrene	0	0	0	0	0	0	0	0	0
	0.1	0.1	0.0	0.0	0.1	0.0	0.2	0.1	0.0
	1.0	0.5	0.0	0.7	1.1	0.4	2.2*	3.2*	0.2
	10.0	5.5*	0.0	10.5*	12.6*	3.2*	11.0*	28.3*	1.5*
Statistics L.S.D.[a,b]		1.3	-	1.7	1.3	1.1	1.4	12.4	1.1
Anthracene	0	0	0	0	0	0	0	0	0
	0.1	2.3*	0.7	0.9	0.9	0.0	1.4	0.1	0.0
	1.0	26.1*	12.9*	9.4*	20.6*	3.0*	26.9*	0.6	0.0
	10.0	266.0*	149.0*	141.0*	261.4*	83.7*	587.2*	36.9*	13.7*
Statistics L.S.D.[a,b]		2.0	1.5	5.3	1.8	1.7	2.1	1.9	1.2
Benz(a)anthracene	0	0	0	0	0	0	0	0	0
	0.1	0.3	0.5	0.0	0.0	0.0	1.3	0.2	0.0
	1.0	4.9*	0.0	0.0	0.0	0.1	10.2	0.5	0.0
	10.0	132.1*	43.3	16.1*	0.0	5.5*	180.7*	18.3*	0.0
Statistics L.S.D.[a,b]		1.8	2.9	12.5	1.1	2.2	2.5	3.4	1.0

[a] Data were $\log_{10}$ transformed for ANOVA after converting all data to $x + 1$.

[b] Least significant difference at $p \leq 0.05$.

*Statistically significant at $p \leq 0.05$.

**Statistically significant at $p \leq 0.10$.

TABLE 38. REGRESSION EQUATIONS OF PLANT UPTAKE OF POLYNUCLEAR AROMATICS

		Chemical			
Plant Species		Anthracene	Benz(a) anthracene	Naphthalene	Phenanthrene
Corn	a	0.108	-0.164	0.089	0.054
	b	1.137	1.056	0.018	0.008
	r^2	0.95	0.95	0.92	0.97
	F	155.4	136.7	92.5	280.7
	Model[a]	2	1	1	1
Fescue	a	-0.050	-0.004	-0.074	N.S.[b]
	b	1.109	0.015	0.85	N.S.
	r^2	0.99	0.92	0.97	N.S.
	F	943.9	100.4	331	N.S.
	Model	2	1	2	N.S.
Immature Soybean Plants	a	-0.028	N.S.	0.020	0.039
	b	0.983	N.S.	0.775	0.010
	r^2	0.90	N.S.	0.94	0.96
	F	69.8	N.S.	152.5	186.6
	Model	2	N.S.	2	1
Mature Soybean Plants	a	-0.043	N.S.	-0.047	-0.135
	b	1.124	N.S.	0.358	0.587
	r^2	0.99	N.S.	0.80	0.92
	F	674.2	N.S.	35.9	105.3
	Model	2	N.S.	2	2
Soybean Seed	a	0.136	-0.019	0.101	-0.082
	b	0.018	0.008	0.848	0.319
	r^2	0.94	0.85	0.95	0.90
	F	132.9	50.6	156.5	85.7
	Model	1	1	2	2
Immature Wheat Plants	a	0.003	-0.028	0.017	-0.056
	b	1.326	1.106	0.653	0.550
	r^2	0.98	0.98	0.94	0.97
	F	42.7	339.3	139.4	275.6
	Model	2	2	2	3
Mature Wheat Plants	a	0.034	0.033	N.S.	-0.059
	b	0.015	0.012	N.S.	0.606
	r^2	0.97	0.94	N.S.	0.59
	F	255.1	104.5	N.S.	14.1
	Model	1	1	N.S.	2

TABLE 38 (continued)

Plant Species		Chemical Anthracene	 Benz(a) anthracene	 Naphthalene	 Phenanthrene
Wheat Seed	a	-0.044	N.S.	0.003	0.016
	b	0.012	N.S.	0.014	0.004
	r^2	0.99	N.S.	0.95	0.98
	F	708.4	N.S.	189.3	421.4
	Model	1	N.S.	1	1
Immature Plants[c]	a	0.004	-0.132	-0.064	-0.070
	b	1.154	0.784	0.814	0.375
	r^2	0.93	0.57	0.92	0.55
	F	543.6	51.6	441.8	50.4
	Model	2	2	2	2
Mature Plants[d]	a	-0.133	-0.050	0.063	-0.094
	b	1.006	0.237	0.206	0.599
	r^2	0.78	0.23	0.23	0.69
	F	72.6	5.4	5.4	44.8
	Model	2	2	2	2
Seeds[d]	a	0.046	-0.019	-0.085	0.023
	b	0.015	0.008	0.800	0.005
	r^2	0.85	0.85	0.79	0.91
	F	117.4	50.6	70.6	206.4
	Model	1	1	2	1

[a] Model: All significant at $p \leq 0.05$.

1. $\log_{10}(\text{ppb dw} + 1) = a + b\,(\text{rate})$.
2. $\log_{10}(\text{ppb dw} + 1) = a + b\,(\log_{10}[\text{rate} + 1])$.

[b] N.S. = not significant at $p \leq 0.05$.

[c] Corn, fescue, wheat, soybean.

[d] Wheat, soybean.

SOIL RETENTION

^{14}C extraction efficiencies ranged from 42% for BA to 12% for NT. AT and PA were 37% and 20%, respectively (Table 36). The GC results generally confirmed the TLC analysis. Values of the precent of ^{14}C as parent compound for GC and TLC analysis were 27.5% and 17.7% for AT, 85.0% and 85.6% for BA, and 62.3% and 54.1% for PA, respectively. The GC value for anthracene was near the minimum level of machine detection.

Significant amounts of PNA's remained in all pots at all rates for each chemical except for naphthalene at 0.1 and 1.0 loading rates (Table 39). Benz(a)anthracene had the highest levels retained followed by phenanthrene, anthraceneand naphthalene.

Leaching into the bottom soil was only significant for naphthalene at the 10 ppm rate for both sets of pots and at 1.0 ppm in corn and wheat pots (Table 40). Benz(a)anthracene leached significantly at the 10.0 ppm rate in corn and wheat pots.

Regression equations were produced to predict soil levels of extractable polynuclear aromatics from pots loaded at 1.0 ppm and sampled at 0, 5, 65, and 123 days. All regressions were significant ($p \leq 0.05$) and predicted soil levels (ppb) by the number of days after loading. Naphthalene showed the most rapid initial decline (Figure 4) at 5 days, only 0.74% of the original NT was extractable from the soil. The best regression equation had a square root term which reflects this rapid initial decline.

$$\text{NT:}\quad \log_{10}(\text{ppb} + 1) = 2.982 - 1.9\,(\log_{10}[\text{Days} + 1])^{1/2}$$

$$r^2 = 0.99 \qquad F = 400.0$$

Anthracene also declined rapidly (Figure 4); only 39.1% of the original anthracene was extractable at 5 days. The best regression equation also had a square root term.

$$\text{AT:}\quad \text{ppb} - 990 - 680\,(\log_{10}[\text{Days} + 1])^{1/2}$$

$$r^2 = 0.99 \qquad F = 1{,}400$$

Phenanthrene decline was more gradual and linear (Figure 4); 78% of the original phenanthrene was extractable by day 5. The best regression equation reflects this linear decay.

$$\text{PA:}\quad \text{ppb} - 1100 - 510\,(\log^{10}[\text{Days} + 1])$$

$$r^2 = 0.96 \qquad F = 190$$

TABLE 39. SOIL RETENTION OF POLYNUCLEAR AROMATICS AFTER FINAL HARVEST--PARTS PER BILLION DRY WEIGHT

Chemical	Rate (mg/kg)	Top Soil			Bottom Soil		
		Initially applied	Corn and wheat pots (123 days)	Soybean and fescue pots (65 days)	Initially applied	Corn and wheat pots (123 days)	Soybean and fescue pots (65 days)
Naphthalene	0	0	0	0	0	0	0
	0.1	50.0	0.1	0.1	0	0.3	0.1
	1.0	970.0	0.5	1.0	0	6.8*	0.5
	10.0	9970.0	5.5*	14.8*	0	91.0*	33.3*
Statistics L.S.D.[a,b]		--	1.7	1.6	-	1.7	2.2
Phenanthrene	0	0	0	0	0	0	0
	0.1	100.0	4.9*	5.0*	0	0.0	0.0
	1.0	1000.0	29.8*	35.2*	0	0.2	0.2
	10.0	10,000.0	223.1*	273.8*	0	0.6	0.8
Statistics L.S.D.[a,b]		--	1.5	3.2	-	1.1	1.9
Anthracene	0	0	0	0	0	0	0
	0.1	85.0	1.9*	3.0	0	0.0	0.0
	1.0	990.0	22.9*	54.8*	0	0.0	0.0
	10.0	9990.0	71.3*	254.7*	0	0.0	0.0
Statistics L.S.D.[a,b]		--	1.4	1.3	-	-	-
Benz(a)anthracene	0	0	0	0	0	0	0
	0.1	98.0	21.5*	43.0*	0	0.0	0.0
	1.0	1000.0	226.9*	301.1*	0	0.6	0.0
	10.0	10,000.0	2981.1*	5169.2*	0	55.2*	0.6
Statistics L.S.D.		--	1.5	2.2	-	6.2	1.7

[a] Data were $\log_{10}$ transformed for ANOVA after converting all data to $x + 1$.

[b] Least significant difference at $p \leq 0.05$.

*Statistically significant at $p \leq 0.05$.

TABLE 40. PERCENT OF ORIGINAL ^{14}C-PNA'S RECOVERED IN BOTTOM SOIL

Chemical	Corn and Wheat Pots (123 days)	Soybean and Fescue Pots (65 days)	Average
Anthracene	0.0	0.0	0.0
Benz(a)anthracene	0.2	0.1	0.1
Naphthalene	1.9	0.6	1.2
Phenanthrene	0.0	0.0	0.0

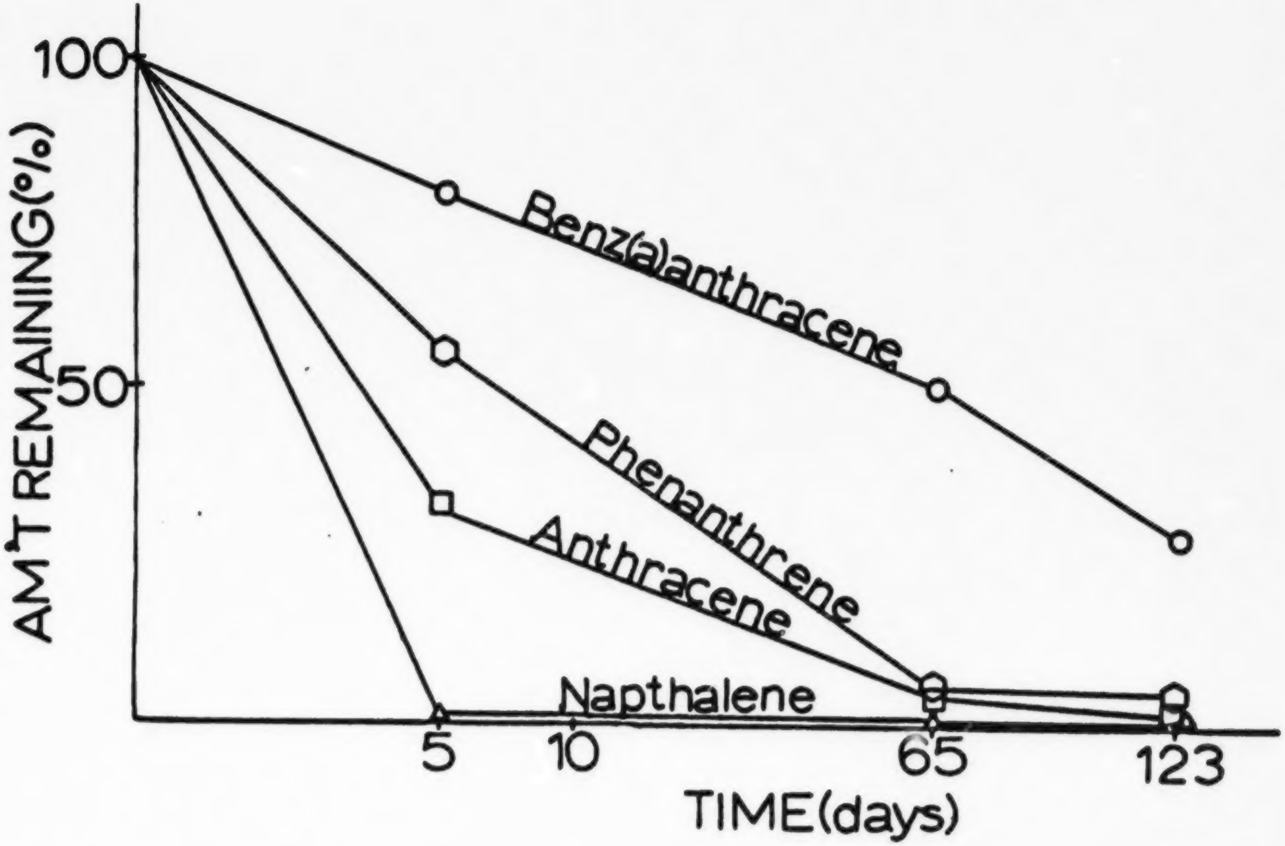

FIG. 4 SOIL RETENTION of POLYNUCLEAR AROMATICS - extractable

Finally, benz(a)anthracene had the most retention in soil (Figure 4); 78% was extractable at 5 days and 23% was extractable at 123 days. The best regression equation was also linear.

$$\text{BA:}\quad \text{ppb} = 1{,}000 - 390\ (\log_{10}[\text{Days} + 1])$$

$$r^2 = 0.95 \qquad F = 140$$

Similar results were obtained from more frequent sampling of smaller pots for BA (Figure 4). These pots showed a less rapid but still linear decline of extractable BA perhaps caused by smaller pot size, fewer plants, or different greenhouse conditions. The half life of BA in smaller pot study was 36 days in comparison to the more complete study with a half life of 23 days.

^{14}C Balance

Only 24% of the naphthalene was recovered in soil and plants by the end of the experiment (Table 41). The remainder had been volatilized or decomposed and released as $^{14}CO_2$. Benz(a)anthracene was more recalcitrant--an average of 80% was accounted for in soil and plant. Intermediate levels of anthracene and phenanthrene were accounted for in soil and plants (averages of 44% and 32%, respectively). Very little of the recovered ^{14}C was in plants (0.0, 0.2, 0.3 and 0.4% for BA, NT, AT and PA, respectively)--soil had essentially all of the radiation at the end of the experiment.

CONCLUSIONS

Plant uptake of the PNA's was in the low ppb range except at the highest loading rate. Uptake was generally only significant at the 10.0 ppm loading rate except for corn and immature wheat plants. Anthracene uptake was generally significant at 1.0 and 10.0 ppm soil loading rate. Since anthracene, benz(a)anthracene and naphthalene uptake exceeded phenanthrene, it appears that plants can take up PNA's with linear configurations easier than nonlinear PNA's. Smaller molecules (anthracene rather than benz(a)anthracene) are also more easily taken up. Naphthalene uptake was less than benz(a)anthracene probably since it was unavailable due to naphthalene's higher volatility. Water solubility appears to be less important than configuration since the water solubility of phenanthrene and naphthalene exceeds anthracene and benz(a)anthracene.

Soil retention reflected chemical structure, water solubility and vapor pressure. Benz(a)anthracene with the largest structure, lowest water solubility and lowest vapor pressure was most resistant to loss. In contrast, naphthalene with the simplest structure, highest vapor pressure and highest water solubility was lost from the pots at the greatest rate. Leaching was significant only for naphthalene which has the highest water solubility.

TABLE 41. TOTAL ^{14}C RECOVERED AT END OF THE EXPERIMENT AND PERCENT OF TOTAL ^{14}C RECOVERED IN ABOVEGROUND PLANT TISSUE

	Total Percent DPM's Recovered			Percent of Total Recovered in Aboveground Plant Tissue		
Chemical	Corn and wheat pots (123 days)	Soybean and fescue pots (65 days)	Average	Corn and wheat pots (123 days)	Soybean and fescue pots (65 days)	Average
Anthracene	30.5	57.8	44.2	0.4	0.2	0.3
Benz(a)anthracene	63.1	96.3	79.7	0.0	0.0	0.0
Naphthalene	20.0	27.6	23.8	0.3	0.2	0.2
Phenanthrene	27.4	36.4	31.9	0.4	0.4	0.4

Plant uptake accounted for very little of the loaded radiation--less than 0.5%. Retention in soil followed water solubility and vapor pressure. The most volatile and water soluble chemical (naphthalene) had less retention than did benz(a) anthracene with the lowest volatility and water solubility. The relatively large amount of ^{14}C-benz(a)anthracene still present at 123 days (63%) indicates concern for long term soil residues.

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SECTION 9

PLANT GROWTH AND UPTAKE AND SOIL RETENTION OF SUBSTITUTED AROMATIC: 14C-CHLOROBENZENE AND THE EFFECT OF SLUDGE

This study was a part of the fifth greenhouse investigation and involved an expanded experimental concept from the previous research phases. A single rate of chemical loading was used along with a control soil. In addition, two municipal sludge application rates were used in conjunction with the single organic chemical loading. Thus, information relative to a control soil was obtained on:

1. the effect of an organic priority pollutant;
2. the effect of municipal sludge in comparison to the pure chemical in a soil in the absence of sludge.

The sludge was aerobic digested sludge from the City of Raleigh. Procedures remained similar to the detailed methodology described previously in this report.

MATERIALS AND METHODS

CHEMICALS

^{14}C labeled chlorobenzene (Amersham CFA 136; Batch 28; Specific Activity 19.7 mCi/mmol) was added to unlabeled chlorobenzene (Fisher reagent grade) in ethanol and sewage sludge and thoroughly mixed into the soil. Sludge was obtained from the Raleigh, NC, Waste Water Treatment Plant from aeration basins. Two replicates of a randomized, complete block design were used with sewage sludge loading rates of 0, 3.2, and 32.1 wet tons/acre (0, 0.16, and 1.63 dry tons/acre), and chlorobenzene loading rate of 9.4 ppb. Two crop combinations were used: A time release fertilizer (Osmocote^{R} 14-14-14) was added to each pot at a rate of 30 gm/pot (14.8 cc/pot) once.

CROP PLANTS

Seven days after chemical loading, two pots from each treatment were planted with 30 wheat (*Triticum aestivum* L. 'Butte') and 10 corn (*Zea mays* L. 'Pioneer 3368A') seeds. Three other pots were planted with 20 soybean [*Glycine max* (L.) Merr. 'Altoona'] and 2.5 cm^3 of fescue (*Festuca arundinacea* Schreb. 'Kentucky 31') seeds. Seeds were planted on October 1,

1982. Height measurements were made and recorded weekly. Corn and fescue plants were harvested at 48 days after the final planting. Soybean plants were thinned and retained for analysis at 36 days and the remaining plants harvested when mature at 80 days. Immature wheat plants were harvested at 41 days; replanted and harvested when mature at 131 days. Wheat and soybean seeds were separated from the nonedible plant portions by hand. Weeds were also harvested and retained for analysis when the final crop was sampled.

CHEMICAL ANALYSIS

About 5 gms of well chopped plant material was placed in a 30 ml centrifuge tube. Twenty ml of hexanes (Fisher pesticide grade) were added and plant tissue was ground on ice in a Tek-Mar homogenizer for 30 seconds. The Tek-Mar shaft was washed with 5 ml of hexanes and tubes centrifuged at 5,000 rpm for 10 minutes. Two ml samples were taken for scintillation counting. Recovery by this method from chlorobenzene injected into corn plants was 88.3%.

Soil samples were Soxhlet extracted in Whatman cellulose thimbles (33mm x 80 mm) in 250 ml of methylene chloride for 8 hours. The filtrate was flash evaporated at 30^{O}C and then reduced to 2 ml under a N_2 stream in a modified (no balls) Snyder column. Samples were subjected to gas chromatographic analysis while two 0.2 $_4$ml aliquots were counted for radiation. Soil samples with known concentrations of chlorobenzene were extracted by this method with 86.7% recovery detectable on the gas chromatograph. All data were adjusted accordingly.

Scintillation cocktail [16.5 gm 2.5-diphenyloxazole (PPO); 0.5 gm 1,4-bis[2-(4-methyl-5-phenyloxozoyl)] benzene (POPOP), 1000 ml Triton X-100 and 2000 ml toluene] was added to each of the vials. A liquid scintillation spectrometer (Packard Tri-Carb Model 2405) was used to assay each vial (two 20-minute counts). Observed radioactivity was corrected for counting efficiency which ranged from 77 to 89%.

Soil samples were oxidized in a Harvey Biological Oxidizer OX-300 for 3 minutes. Samples were then counted to determine ^{14}C extraction efficiency. Harvey ^{14}C cocktail was used to trap the $^{14}CO_2$.

Standards were analyzed on a Varian 3700 Gas Chromatograph with E.C.D. on a 4 mm i.d. by 6 foot glass column packed with 1.5% OV 17/1.95% QFI on 100/120 mesh Chromosorb (T-detector = 3000C, T-injector = 250^{O}C). Column temperature was programmed at 30^{O}C for 14 minutes and then 25^{O}C/minute to 160^{O}C for 1 minute. Carrier gas was N_2 at 32 ml/minute. Retention time was 12.2 minutes. The minimum level of detection was 100 ng/ml or 6.7 ppm soil and 80 ppm plant. Since ^{14}C extracts had much lower concentrations, no GC confirmational analysis was performed.

Soil samples were sent to N. C. Department of Agriculture lab for chemical nutrient and heavy metal analysis before and after the experiment. Percent organic matter was determined by combusting dry soil samples at 400°C for 8 hours.

CHEMICAL CONCENTRATION CALCULATIONS

Concentrations of ^{14}C chlorobenzene in plants and soil were calculated by subtracting background counts (from control samples); multiplying by 12.5 for plants (to account for 2 ml from 25 ml samples) or by 10 for soil (0.2 ml of 2 ml) and dividing by sample dry weight to yield ^{14}C activity in disintegrations per minute per gram of dry weight (dpm/gdw). A conversion factor was calculated by dividing the activity of the ^{14}C chlorobenzene by the total chlorobenzene added [(mg ^{14}C x dpm/mg ^{14}C)/(mg ^{14}C + mg ^{12}C]. The value was divided by extraction efficiency and multiplied by the dpm/gdw to yield parts per million dry weight.

Total harvest dry weight for each crop was multiplied by the crop's dpm/gdw for each pot. These were summed to determine total aboveground radiation recovered per pot. Total dpm's found in aboveground plants and soil were summed to determine a mass balance for ^{14}C.

STATISTICAL ANALYSIS

Data were analyzed with the General Linear Model (GLM) program from SAS Variables used in the ANOVA were replicate and sludge rate. Presence of chlorobenzene was used for plant growth analysis. Plant height data were also analyzed over time (2, 4 and 6 weeks for corn and fescue and also 12 and 18 weeks for soybean and wheat).

RESULTS

PLANT GROWTH

Sewage sludge or chlorobenzene had no effect on plant height (Table 42). For instance, at harvest, fescue grown without chlorobenzene averaged 23 cm in height while fescue with chlorobenzene averaged 24 cm tall. Corn height showed some differences - corn in chlorobenzene pots averaged 62 cm tall while that in pots without chlorobenzene averaged 53 cm tall. Differences were caused by the presence of chlorobenzene.

Plant dry weight at harvest was more affected by sludge and/or chlorobenzene (Table 43). Corn and mature soybean plants weight was enhanced while fescue weight was reduced. The fescue weight reduction was caused by sludge rate while the enhanced growth of corn and mature soybean plants was due to chlorobenzene presence.

TABLE 42. HEIGHT (cm) OF PLANTS AT HARVEST GROWN IN GREENHOUSE POTS WITH CHLOROBENZENE AND SEWAGE SLUDGE

Sludge Rate wet tons/acre	Corn	Fescue	Immature Soybean Plant	Mature Soybean Plant	Immature Wheat Plant	Mature Wheat Plant
Controls						
0	51 (4)[a]	17 (3)	43 (7)	49 (8)	29 (6)	50 (1)
3.2	55 (4)	25 (4)	40 (0)	49 (0)	29 (8)	43 (6)
32.0	54 (6)	26 (3)	40 (2)	48 (1)	36 (1)	49 (7)
Chlorobenzene						
0	59 (1)	24 (4)	42 (4)	48 (1)	31 (1)	49 (0)
3.2	60 (0)	24 (4)	51 (1)	57 (10)	30 (4)	50 (4)
32.0	58 (1	24 (1)	45 (0)	46 (2)	29 (6)	41 (4)
Statistics						
L.S.D. ($p \leq .05$)	8	8	10	14	13	13
F	2.50	2.29	1.12	0.71	0.25	0.32
Source of F						
Sludge rate	0.46	2.09	0.48	1.33	0.36	0.56
Presence chlorobenzene	8.84**	0.66	3.36	0.20	0.18	0.01

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* Statistically significant at $p \leq 0.05$

** Statistically significant at $p \leq 0.10$

[a] Height in cm (standard deviation)

TABLE 43. DRY WEIGHT (gm) PER POT OF PLANTS AT HARVEST GROWN IN GREENHOUSE POTS WITH CHLOROBENZENE AND SEWAGE SLUDGE

Sludge Rate wet tons/acre	Corn	Fescue	Immature Soybean Plants	Mature Soybean Plants	Soybean Seed
Controls					
0	15.51	3.74	5.96	8.80	4.46
	(0.23)[a]	(0.23)	(1.15)	(0.83)	(1.61)
3.2	17.54	4.30	4.64	10.88	4.22
	(2.46)	(0.54)	(0.28)	(0.62)	(1.66)
32.0	17.11	4.54	5.46	11.60	3.05
	(0.57)	(0.63)	(0.22)	(1.24)	(0.38)
Chlorobenzene					
0	21.28	3.24	4.64	7.58	5.27
	(2.86)	(0.26)	(0.28)	(0.71)	(0.61)
3.2	21.08	4.38	5.61	9.66	5.46
	(2.42)	(0.14)	(0.74)	(3.91)	(0.08)
32.0	22.98	3.86	4.29	7.72	6.40
	(2.44)	(0.23)	(0.06)	(0.66)	(1.51)
Statistics					
L.S.D. ($p \leq .05$)	4.65	0.97	2.05	3.67	3.07
F	5.50**	3.09*	0.39	3.01*	1.46
Source of F					
Sludge Rate	0.71	4.95**	0.23	1.89	0.01
Presence of Chlorobenzene	19.86***	2.40	1.06	5.53*	5.76**

* Statistically significant at $p<0.10$
** Statistically significant at $p \leq 0.05$
*** Statistically significant at $p \leq 0.01$

[a] Average weight in gm (standard deviation)

TABLE 43 (continued)

Sludge Rate wet tons/acre	Immature Wheat Plant	Mature Wheat Plant	Wheat Seed
Controls			
0	1.12 (0.64)	5.54 (0.33)	1.16 (0.64)
3.2	0.89 (0.14)	2.34 (0.18)	1.17 (0.81)
32.0	1.92 (0.45)	3.27 (0.91)	2.20 (0.28)
Chlorobenzene			
0	1.89 (0.14)	4.08 (1.00)	2.38 (1.36)
3.2	1.62 (0.14)	5.67 (1.58)	3.08 (0.35)
32.0	1.60 (0.15)	3.44 (0.54)	1.70 (0.77)
Statistics			
L.S.D. ($p \leq .05$)	1.08	3.63	2.20
F	1.16	0.61	0.96
Source of F			
Sludge Rate	1.20	0.90	0.15
Presence of Chlorobenzene	2.24	0.59	2.69

Examination of plant growth (Table 44) revealed that sludge reduced the initial growth of all plants except fescue. This effect was gone after two weeks.

PLANT UPTAKE

Average extractable ^{14}C chlorobenzene ranged from zero to 45.8 ppb (Table 45). Immature wheat and soybean plants had no extractable ^{14}C while corn averaged 0.05 ppb and fescue averaged 11.7 ppb. Weeds were somewhat higher with average extractable ^{14}C of 25.2 ppb. Mature plants and seeds have not yet been extracted.

SOIL RETENTION

Sludge had little apparent effect on soil properties such as percent organic matter, cation exchange capacity, pH or percent base saturation (Table 46). Zinc concentrations were elevated in sludge pots while manganese and copper levels were somewhat higher in pots with 32 tons/acre of sludge.

^{14}C chlorobenzene was rapidly lost from the soil (Table 47). At 48 days, an average of 8% of the radiation was still in the soil. This decreased further to 6.6% left by 80 days. Plant uptake accounted for less than 0.01 percent of the applied radiation. Therefore, about 93.4% of the ^{14}C chlorobenzene either was degraded or volatilized. There was no significant difference between 40 and 80 day soils.

CONCLUSIONS

Except for wheat and soybean seeds, chlorobenzene has little apparent effect on crop plant height or dry weight when loaded at 9.4 ppb (100 times normal sewage sludge concentration). There was also no apparent effect of sewage sludge on growth or ^{14}C uptake at 3.2 and 32 tons/acre nor an interactive effect of sludge and chlorobenzene. Plant growth was initially slowed by sludge but this effect was gone by four weeks. Extractable ^{14}C reported as chlorobenzene was extremely low (less than 100 ppb) in plant tissue and only present in plants harvested earliest. Soil levels of ^{14}C chlorobenzene decreased extremely rapidly to 8% by 40 days.

TABLE 44. STATISTICAL SIGNIFICANCE AND SOURCES OF SIGNIFICANCE OF HEIGHT (cm) OF PLANTS IN CHLOROBENZENE AMENDED POTS

Crop	Height (weeks)				
	2	4	6	12	18
Corn	*** (S,C)	n.s.	n.s.	n.d.	n.d.
Fescue	n.s.	n.s.	n.s.	n.d.	n.d.
Immature Soybean	* (S)	n.s.	n.s.	n.d.	n.d.
Immature Wheat	* (S)	n.s.	n.s.	n.d.	n.d.
Mature Wheat	* (S)	n.s.	n.s.	n.s.	n.s.

n.s. = not significant
n.d. = no data

Sources of significance
S = sludge rate
C = presence of chlorobenzene

* Statistically significant at $p<0.10$
** Statistically significant at $p\leq0.05$
*** Statistically significant at $p\leq0.01$

TABLE 45. PLANT UPTAKE OF EXTRACTABLE ^{14}C CHLOROBENZENE IN PLANTS GROWN IN GREENHOUSE POTS - ppb.*

					Weeds	
Sludge tons/ac.	Corn	Fescue	Immature Wheat Plant	Immature Soybean Plant	Soybean Pots	Wheat Pots
0	0.06 (0.05)	22.60 (11.53)	0	0	5.2	37.6
3.2	0.08 (0.08)	11.60 (0.85)	0	0	8.8	0
32.0	0.02 (0.00)	0.90 (1.27)	0	0	2.1	100.0
Statistics						
Average	0.05	11.70	0	0	4.6	45.8
F	0.26	0.90	-	-		

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* results only for ^{14}C

TABLE 46. SOIL NUTRIENTS, HEAVY METALS AND pH OF CONTROL POTS

Time (days)	Sludge tons/ac.	Percent Organic matter	CEC meq/100cm^3	Percent base saturation	pH	Indices		
						Mn	Zn	Cu
80	0	1.98	3.7	57	4.8	32	64	30
	3.2	2.06	3.2	62	5.2	29	214	34
	32.0	2.22	3.6	66	5.2	51	280	50
131	0	2.03	4.2	52	4.8	39	96	42
	3.2	2.20	3.7	46	4.9	30	184	44
	32.0	2.11	4.6	56	4.9	67	260	59

TABLE 47. OXIDIZABLE ^{14}C CHLOROBENZENE FROM SOIL IN GREENHOUSE POTS - PPB REMAINING AND PERCENT OF ORIGINAL APPLIED RADIATION LEFT***

		^{14}C - ppb		% of Original Radiation Left	
Time	Sludge Rate tons/acre	Soybean-fescue pots	Corn-Wheat pots	Soybean-fescue pots	Corn-Wheat pots
40 days					
	0	0.8	0.7	8.3%	7.7%
	3.2	0.9	0.8	10.0	9.2
	32.0	0.6	0.7	6.2	7.4
Average		0.8	0.7	8.5%	7.4%
Final harvest*					
	0	0.8	ND**	6.2%	ND
	3.2	0.6	ND	7.4	ND
	32.0	0.7	ND	6.4	ND
Average		0.7		6.6%	

Statistics (all data)

F 0.10

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* Corn-wheat pots = 131 day
Soybean-fescue pots = 80 day

** ND = no data. Samples contaminated during extraction.

*** results only for ^{14}C

SECTION 10

PLANT GROWTH, UPTAKE AND SOIL RETENTION OF SUBSTITUTED AROMATICS: PARADICHLOROBENZENE AND THE EFFECT OF SLUDGE

This study was a part of the fifth greenhouse investigation and involved the same protocol as that of chlorobenzene. The same sludge was used for these experiments, following the procedures described earlier in this report.

MATERIALS AND METHODS

CHEMICALS

^{14}C labeled paradiclorobenzene (Amersham CFQ 2443, Batch C/58; Specific Activity 16.1 mCi/mmol) was added to unlabeled *p*-dichlorobenzene (Fisher reagent grade) in ethanol and sewage sludge and thoroughly mixed into the soil. Sludge was obtained from the Raleigh, N.C., Waste Water Treatment Plant from aeration basins. Two replicates of a randomized, complete block design were used with sewage sludge loading rates of 0, 3.2, and 32.1 wet tons/acre (0, 0.16, and 1.63 dry tons/acre), and *p*-dichlorobenzene loading rate of 0.2 ppm. Two crop combinations were used.

CROP PLANTS

Seven days after chemical loading, two pots from each treatment were planted with 30 wheat (Triticum aestivum L. 'Butte') and 10 corn (*Zea mays* L. 'Pioneer 3368a') seeds. Three other pots were planted with 20 soybean [*Glycine max* (L.) Merr. 'Altoona'] and 2.5 cm^3 of fescue (*Festuca arundinacea* Schreb. 'Kentucky 31') seeds. Seeds were planted on October 20, 1982. Height measurements were made and recorded weekly. Corn and fescue plants were harvested at 59 days after the final planting. Soybean plants were thinned and retained for analysis at 53 days and the remaining plants were harvested when mature at 102 days. Immature wheat plants were harvested at 53 days; replanted and harvested when mature, at 129 days. Wheat and soybean seeds were separated from nonedible plant portions by hand. Weeds were also harvested and retained for analysis when the final crop was sampled.

CHEMICAL ANALYSIS

Extraction methods were modified from Erickson et al. (1981) and Harrold and Young (1982). Plant samples were homogenized for one minute with a "45" Virtis homogenizer at 39,000 RPM in 50 ml of methylene chloride. Soil samples were weighed and then transferred to cellulose thimbles for extraction. The plant material was then transferred to cellulose extraction thimbles (Whatman 33 mm x 80 mm) and Soxhlet extracted in 250 ml of methylene chloride for 8 hours. The filtrate was flash evaporated at 30°C and then reduced to 2 ml under a N_2 stream in a modified (no balls) Snyder column. One ul sample was subjected to gas chromatographic analysis while 200 ul samples were counted for radiation. Soil and plant samples with known concentrations of p-dichlorobenzene were extracted by this method with 48.8% recovery detectable on the gas chromatograph. All data were adjusted accordingly.

Scintillation cocktail [16.5 gm 2.5-diphenyloxazole (PPO); 0.5 gm 1,4-bis[2-(4-methyl-5-phenyloxozoyl)] benzene (POPOP), 1000 ml Triton X-100 and 2000 ml toluene] was added to each of the vials. A liquid scintillation spectrometer (Packard Tri-Carb Model 2405) was used to assay each vial (two 20-minute counts). Observed radioactivity was corrected for counting efficiency which ranged from 77% to 89%.

Soil samples were oxidated in a Harvey Biological Oxidizer OX-300 for 3 minutes. Samples were then counted to determine ^{14}C extraction efficiency. Harvey ^{14}C cocktail was used to trap the $^{14}CO_2$. Soil extraction efficiency was 77%.

Samples were analyzed on a Varian 3700 Gas Chromatograph with E.C.D. on a 4 mm i.d. by 6 foot glass column packed with 1.5% OV 17/1.95% QFI on 100/120 mesh Chromosorb (T-detector = 300°C, T-injector = 250°C). Column temperature was programmed at 60°C for 13 minutes and then 250°C/minute to 160°C for 1 minute. Carrier gas was N_2 at 32 ml/minute.

The minimum level of detection was 200 pg/ul. Retention time was 10.5 minutes. Known concentrations of p-dichlorobenzene were injected with control soil and plant samples to determine retention time and machine calibration. The E.C.D. was cleaned daily by using H_2 gas (60 ml/min) with T-detector = 350°C for 30 to 60 minutes. E.C.D. sensitivity was checked daily.

Soil samples were sent to N. C. Department of Agriculture lab for chemical nutrient and heavy metal analysis before and after the experiment. Percent organic matter was determined by combusting dry soil samples at 400°C for 8 hours.

CHEMICAL CONCENTRATION CALCULATIONS

Concentrations of ^{14}C p-dichlorobenzene in plants and soil were calculated by subtracting background counts (from control samples); multiplying by 10 (to account for 200 ul from 2 ml samples) and dividing by sample dry weight to yield ^{14}C activity in disintegrations per minute per gram of dry weight (dpm/gdw). A conversion factor was calculated by dividing the activity of the ^{14}C p-dichlorobenzene by the total p-dichlorobenzene added [(mg ^{14}C x dpm/mg ^{14}C)/(mg ^{14}C + mg ^{12}C]. The value was divided by chemical extraction efficiency (48.8%) and multiplied by the dpm/gdw to yield parts per million dry weight.

Total harvest dry weight for each crop was multiplied by the crop dpm/gdw for each pot. These were summed to determine total aboveground extractable radiation recovered per pot. Total dpm found in aboveground plants and soils were summed to determine mass balance for extractable ^{14}C.

STATISTICAL ANALYSIS

Data were analyzed with the General Linear Model (GLM) program from SAS (Barr et al., 1977). Variables used in the ANOVAs were replicate and sludge rate. The presence of p-dichlorobenzene was included in plant growth ANOVAS. Plant height data were also analyzed over time (2, 4, and 6 weeks for corn and fescue and also 12 and 18 weeks for soybean and wheat).

RESULTS

PLANT GROWTH

In general, there was little effect of sludge or p-dichlorobenzene on plant height (Table 48). Exceptions were enhanced corn height at 3.2 tons/acre of sludge and decreased immature wheat height at 3.2 tons/acre of sludge. Mature wheat plant showed a slight reduction of height from p-dichlorobenzene. At harvest, corn plants grown without p-dichlorobenzene averaged 72 cm while those in p-dichlorobenzene amended pots averaged 67 cm. Fescue averaged 30 cm in pots without p-dichlorobenzene and 25 cm amended pots. Mature wheat plants averaged 47 cm in control pots and 39 cm in p-dichlorobenzene pots. Mature soybean plants averaged 67 cm in control pots and 65 cm in p-dichlorobenzene pots. The high sludge rates slowed the initial growth of soybeans but had little effect on other plants and by four weeks, there was little difference in soybean height.

TABLE 48. HEIGHT (cm) AND STANDARD DEVIATION OF PLANTS AT HARVEST GROWN IN GREENHOUSE POTS WITH *p*-DICHLOROBENZENE

Sludge Rate, wet tons/acre	Corn	Fescue	Immature Soybean Plant	Mature Soybean Plant	Immature Wheat Plant	Mature Wheat Plant
Controls						
0	62 (2)[a]	24 (6)	39 (2)	66 (8)	30 (4)	52 (4)
3.2	76 (2)	32 (4)	33 (4)	70 (.)	18 (4)	43(11)
32.1	78 (2)	34 (5)	36 (4)	64 (6)	34 (6)	46 (1)
p-Dichlorobenzene						
0	60 (0)	24 (4)	35 (1)	51(13)	30 (0)	37 (8)
3.2	71 (8)	24 (6)	38 (0)	66 (6)	22 (6)	43 (1)
32.1	71 (8)	28 (4)	32 (6)	77 (4)	36 (6)	37 (3)
Statistics						
L.S.D.($p \leq 0.05$)	12	11	10	26	9	15
F	5.58***	1.46	0.39	0.79	8.16***	1.77
Source F						
Sludge rate	9.74***	1.70	0.60	1.43	14.76***	0.16
p-Dichlorobenzene presence	2.80	2.44	0.12	0.13	1.19	5.06**

** Significant at $p \leq 0.05$
*** Significant at $p \leq 0.01$
(.) Only one plant remaining
[a] Height in cm (standard deviation)

Average dry weight was generally not affected by sludge or p-dichlorobenzene (Table 49). Immature soybean plants were an exception - sludge presence caused a weight reduction. Soybean seed weight was also reduced by p-dichlorobenzene. Immature wheat dry weight was weakly affected by sludge rate with weight reductions at 3.2 tons/acre.

PLANT UPTAKE

Extractable ^{14}C was taken up in all plants except wheat and soybean seed (Table 50). Sludge rate had no statistically significant effect. Average uptake was 91 ppb in corn, 1387 in fescue, 1056 ppb in immature wheat, 348 ppb in immature soybean, 153 ppb in mature wheat plants, 342 ppb in mature soybean plants and zero for wheat and soybean seed.

The ^{14}C extracted was above the minimum level of detection for green soybeans (no sludge) and mature soybean (3.2 and 32 tons/acre of sludge). No p-dichlorobenzene was detected in these samples. Minimum levels of detection averaged 400 ppb for all plants.

SOIL RETENTION

Nutrients and Heavy Metals

Additions of sludge caused little change in soil nutrient levels (P,K, cation exchange capacity, percent base saturation), soil pH or soil organic matter (Table 51). Manganese and copper were elevated at the high sludge rate while zinc was higher than control levels at all sludge rates. Soil sampled at 90 days was similar to 151 day soil.

^{14}C paradichlorobenzene

^{14}C disappeared rapidly from the pots (Table 52). By 49 days, only 4.4% of the label was present. This further decreased to 3.2% by 90 days and 2.6% by 151 days. There was no apparent effect of sludge rate on soil retention of ^{14}C.

No statistically significant difference was detected between date of soil harvest.

Gas Chromatographic analysis of soil extracts from 90 days revealed that the label left was not p-dichlorobenzene. An average of 4.6 ppb ^{14}C was present and the GC minimum level of detection was 2.8 ppb.

TABLE 49. DRY WEIGHT (gm) AND STANDARD DEVIATION OF PLANTS GROWN IN GREENHOUSE POTS WITH p-DICHLOROBENZENE

Sludge Rate, wet tons/acre	Corn	Fescue	Immature Soybean Plant	Mature Soybean Plant	Soybean Plant
Controls					
0	21.22	1.49	8.71	11.98	5.05
	(5.08)[a]	(0.41)	(1.53)	(5.49)	(0.24)
3.2	22.41	2.35	6.20	11.77	6.03
	(2.73)	(1.53)	(3.86)	(3.55)	(1.87)
32.1	23.64	2.20	5.20	9.90	4.81
	(1.59)	(0.87)	(0.22)	(.)	(.)
p-Dichlorobenzene					
0	14.32	1.71	7.51	9.38	2.02
	(0.92)	(0.62)	(1.87)	(0.99)	(1.35)
3.2	22.69	1.54	6.77	12.52	2.75
	(4.53)	(0.59)	(0.93)	(1.07)	(0.69)
32.1	18.73	2.02	3.58	13.67	2.38
	(6.42)	(0.01)	(1.39)	(3.97)	(.)
Statistics					
L.S.D. ($p \leq .05$)	9.28	1.81	3.90	7.79	2.82
F (overall)	1.80	0.49	3.38*	0.57	5.04**
Source F					
Sludge rate	1.57	0.46	5.12**	0.25	1.02
p-Dichlorobenzene presence	2.88	0.34	0.62	0.08	18.09**

* Significant at $p \leq 0.01$

** Significant at $p \leq 0.05$

(.) Only one plant remaining

[a] Dry weight in gm (standard deviation)

TABLE 49 (continued)

Sludge Rate, wet tons/acre	Immature Wheat Plant	Mature Wheat Plant	Wheat Seed
Controls			
0	0.98 (0.08)	5.16 (1.32)	1.48 (0.48)
3.2	0.39 (0.04)	4.69 (1.69)	1.73 (0.14)
32.1	1.49 (0.36)	5.26 (0.60)	1.92 (1.03)
p-Dichlorobenzene			
0	1.29 (0.24)	4.07 (0.54)	0.94 (0.21)
3.2	0.73 (0.45)	4.68 (0.08)	1.69 (0.01)
32.1	1.19 (0.56)	4.67 (1.95)	1.98 (0.62)
Statistics			
L.S.D. ($p \leq .05$)	0.88	2.46	1.18
F (overall)	2.43	0.77	1.43
Source F			
Sludge rate	4.69*	0.13	2.28
p-Dichlorobenzene presence	0.31	0.89	0.37

* Significant at $p \leq 0.10$

TABLE 50. UPTAKE AND STANDARD DEVIATIONS (ppb) OF EXTRACTABLE ^{14}C *p*-DICHLOROBENZENE IN PLANTS GROWN IN GREENHOUSE POTS

Sludge, tons/ac.	Corn	Fescue	Immature Soybean Plants	Mature Soybean Plants	Soybean Seed	Immature Wheat Plants	Mature Wheat Plants	Wheat Seed
0	86	1354	552	104	0	1007	94	0
	(91)	(177)	(11)	(30)		(.)	(14)	
3.2	68	1250	283	495	0	(.)	146	0
	(52)	(.)	(112)	(488)		(.)	(71)	
32	120	1488	208	426	0	1107	218	0
	(47)	(500)	(4)	(11)		(.)	(122)	
Statistics								
Average	91	1387	348	342	0	1056	153	0
L.S.D. ($p \leq .05$)	159	2041	540	654	-	1696	255	-
F	0.21	0.16	0.41	0.50	-	0.17	0.23	-

* Significant at $p \leq 0.1$

(.) Only one plant remaining

TABLE 51. SOIL NUTRIENTS, HEAVY METALS AND pH OF CONTROL POTS

Time (days)	Sludge tons/ac.	Percent Organic Matter	CEC (meq/100 cm^3)	Percent Base Saturation	Indices pH	Mn	Zn	Cu
	0	1.18	3.0	72	5.1	18	90	28
90	3.2	1.29	2.8	71	5.0	20	159	30
	32.1	1.24	4.3	67	5.2	52	250	55
	0	1.12	3.2	62	5.2	20	102	72
150	3.2	1.23	1.9	59	5.0	18	132	45
	32.1	1.38	3.0	66	5.4	56	200	118

TABLE 52. SOIL CONCENTRATIONS OF ^{14}C AS p-DICHLOROBENZENE(1)

Time	Sludge ton/acre	Percent Original left (2)	ppb(3)
49 days	0	4.5%	9.0
	3.2	3.2	6.5
	32.0	5.3	10.5
90 days	0	3.0	6.0
	3.2	2.9	5.8
	32.0	3.7	7.4
151 days	0	2.4	4.8
	3.2	2.2	4.6
	32.0	3.1	6.2
Statistics			
F	0.36		
Source of F Sludge Rate	0.28		
Time	0.60		

---

(1) GC Analysis revealed the label left was not p-dichlorobenzene
(2) From oxidized soil samples
(3) Original loading rate = 200 ppb.

^{14}C Mass Balance

Most of the ^{14}C was volatilized or decomposed and released as $^{14}CO_2$ (Table 53). Plants accounted for an average of 0.9% of the radiation while soil accounted for 2.6%.

CONCLUSIONS

p-Dichlorobenzene had little effect on crop plant height or dry weight when loaded at 0.2 ppm (100 times rate found in sewage sludge). There was no effect of sewage sludge on ^{14}C uptake at 3.2 and 32 tons/acre. Extractable ^{14}C p-dichlorobenzene was greater than 1 ppm for young plants and less than 500 ppb for mature plants. Corn was an exception to this rule. Wheat and corn seed had no extractable ^{14}C. Soil levels of ^{14}C decreased extremely rapidly to 4.4% by 49 days and 2.6% by 151 days.

REFERENCES

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TABLE 53. MASS BALANCE OF EXTRACTABLE ^{14}C FROM p-DICHLOROBENZENE APPLIED IN GREENHOUSE POTS - PERCENT OF ORIGINAL APPLIED ^{14}C

Sludge tons/acre	Percent of Original In Plants	In Soil	In Plants and Soil
0	1.5	2.0	3.5
0	0.9	2.6	3.5
3.2	0.8	1.4	2.2
3.2	1.0	3.0	4.0
32.0	0.7	2.9	3.6
32.0	0.7	3.8	4.5
Avg. Percent	0.9	2.6	3.5

SECTION 11

PLANT GROWTH, UPTAKE AND SOIL RETENTION OF DI-N-BUTYL PHTHALATE ACID ESTER AND THE EFFECT OF MUNICIPAL SLUDGE

This study was a part of the fifth greenhouse investigation and involved the same protocol as that of chlorobenzene and p-dichlorobenzene. The same sludge was used for these experiments, following the procedures described earlier in this report.

MATERIALS AND METHODS

CHEMICALS

^{14}C labeled Di-n-butyl phthalate (DnBP) was mixed with unlabeled DnBP (Fisher reagent grade) and added to the soil at 0.22 ppm (1998 dpm/gdw) along with sludge at eight rates (0, 3, 6, 9, 18, 32, 64 and 128 wet tons/acre).

The mixtures of ^{14}C labeled and DnBP were thoroughly mixed into the soil of each pot. A sample of each chemical mixture was retained and analyzed to confirm loading rates. Sludge was obtained from aeration basins from the Raleigh (N.C.) Municipal Wastewater Treatment Plant. Two replicates of a randomized, complete block design was used with two control pots. A total of 72 pots were used.

CROP PLANTS

On April 8, 1983, one week after chemical loading, each pot was planted with six soybean [Glycine max (L.) Merr.] 'Altoona' seeds. Height measurements were made weekly. Plants were harvested at 28 and 73 days when their respective pots were harvested. Weeds were harvested from pots at 73 days.

CHEMICAL ANALYSIS

Plant samples were homogenized for 2 to 3 minutes with a "45" Virtis homogenizer at approximately 39,000 rpm. Hexane:acetone (1:1-Fisher H-300 pesticide grade and A-18 certified grade) were used for each sample.

In chemical extraction from the soil, a 30 gram sample was weighed, placed in a 600 ml plastic beaker with 50 ml of hexane:acetone (1:1) and ground with a Tekmar Homogenizer for two minutes. After homogenization, the blades were washed into the grinding flask and flask contents were filtered through a Whatman GF/B 2.4 cm glass Microfiber filter under suction into a side-armed Erlenmeyer flask. The flask was washed into a 250 ml beaker with hexane:acetone and placed under a hood. When evaporated to about 5 ml, the filtrate was washed into a glass scintillation vial and dried to about 2 ml. Several drops of sodium hypochlorite bleach (Clorox^R) were added to the vials to bleach out chlorophyll.

Scintillation cocktail [16.5 gm 2,5 diphenyloxazole (PPO); 0.5 gm 1,4-bis [2-(4-methyl-5-phenyloxozoyl)] benzene (POPOP); 1000 ml Triton X-100 and 2000 ml toluene] was added to each vial. A liquid scintillation spectrometer (Packard Tri-Carb Model 2405) was used to twice assay each vial (10 minutes or 20,000 counts). Observed radioactivity was corrected for counting efficiency which ranged from 73 to 85%.

Plant samples (after extraction) and soil samples were oxidized in a Harvey Biological Oxidizer OX-300 for 3 minutes to determine amounts of ^{14}C bound (unextractable) residue. Harvey ^{14}C cocktail was used to trap the $^{14}CO_2$.

CHEMICAL CONCENTRATION CALCULATIONS

Concentrations of ^{14}C DnBP in plants and soil were calculated by subtracting background counts (from control samples); dividing by sample dry weight to yield ^{14}C activity in disintegrations per minute per gram of dry weight (dpm/gdw). A conversion factor was calculated by dividing the activity of the ^{14}C DnBP by the total DnBP added [(mg ^{14}C x dpm/mg ^{14}C)/(mg ^{14}C + mg ^{12}C]. The value was divided by chemical extraction efficiency (91.1% for plant and 78.5% for soil) and multiplied by the dpm/gdw to yield parts per million dry weight. Total ^{14}C plant uptakes were the sum of extractable ^{14}C (dpm/gdw) and oxidizable ^{14}C (dpm/gdw).

STATISTICAL ANALYSES

Data were analyzed with the General Linear Model (GLM) program from SAS Variables used in the ANOVAs were replicate and sludge rate. Presence of DnBP was also used for plant growth analysis while time was used for soil analysis. Stepwise regression was used to analyze soil retention data.

RESULTS

SOIL

Soil disappearance of extractable ^{14}C DnBP was significantly affected by sludge rate and time when all dates were considered (Figure 5, Table 54). This relationship disappeared when each date was analyzed separately. There was little difference at any sludge rate between 7 and 14 days but by 28 days, soil disappearance accelerated. The 73 day soil showed further ^{14}C DnBP degradation but at a lower rate than between 14 and 28 days. Soil disappearance increased with increasing sludge rate (Figure 5) with most noticeable increased disappearance between 6 and 18 tons/acre. By 73 days, there was little difference between sludge rates. Soil disappearance of total ^{14}C DnBP showed similar patterns (Figure 6). An average of 75% of the ^{14}C DnBP was extractable in the 7, 14 and 28 day samples while 50% of the ^{14}C was extractable at 73 days (Table 55). There was little apparent effect on sludge rate on soil ^{14}C extraction efficiency.

The regression equation which best predicted extractable ^{14}C DnBP concentration was:

$$\text{Extractable } ^{14}C \text{ (ppb)} = 839.88 - 8.62 \text{ (number of days)} - 1.40 \text{ (sludge tons/acre)}$$

$$R^2 = 0.41$$

Date had the largest F value (44.94; $p \leq .0001$) while sludge rate had a smaller effect ($F=2.87$, $p \leq 0.095$). Total ^{14}C DnBP was best predicted as below:

$$\text{Total } ^{14}C \text{ (ppb)} = 1382.95 - 12.47 \text{ (number of days)} - 1.40 \text{ (sludge tons/acre)}$$

$$R^2 = 0.32$$

Again, date had the largest effect on the equation ($F=30.84$; $p \leq .0001$) while sludge rate had less effect ($F=0.95$; $p \leq .3337$).

FIGURE 5

Percent of Original 14C-DnBP Extractable from Soil

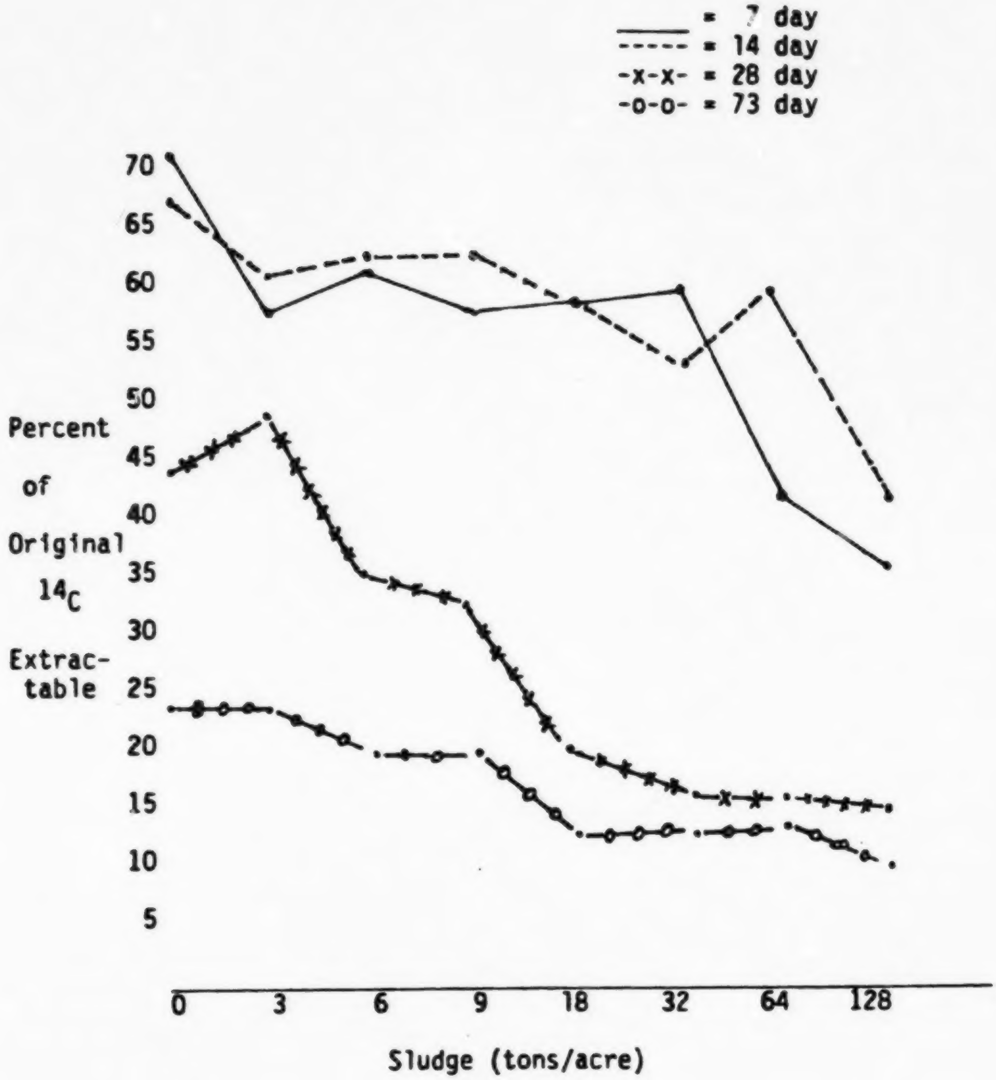

TABLE 54. SUMMARY OF STATISTICAL SIGNIFICANCE OF ^{14}C DnBP (extracted and total) IN SOIL

	Extracted - ^{14}C	Total - ^{14}C
All dates		
F	8.17***	7.50***
Source of F:		
Date	24.01***	20.89***
Sludge Rate	2.54**	2.80**
7 day		
F	0.45	0.71
Source of F - sludge	0.51	0.80
14 day		
F	0.52	0.40
Source of F - sludge	0.59	0.44
28 day		
F	1.17	1.17
Source of F - sludge	1.34	1.32
73 day		
F	0.72	0.54
Source of F - sludge	0.82	0.60

** Statistically significant at $p \leq 0.05$
*** Statistically significant at $p \leq 0.01$

FIGURE 6

Percent of Original 14C - DnBP Oxidizable from Soil

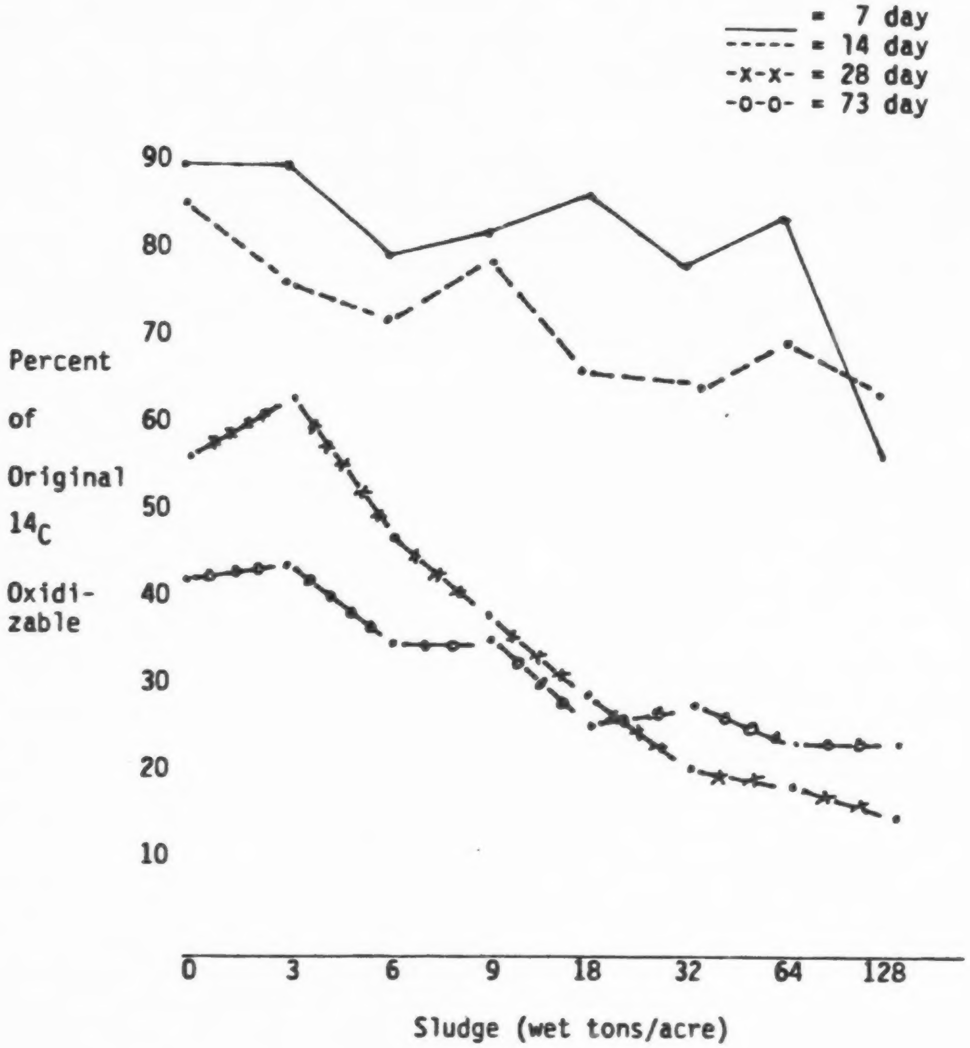

TABLE 55. SOIL ^{14}C EXTRACTION EFFICIENCY

$$\frac{\text{dpm/gdw extractions}}{\text{dpm/gdw oxidations}}$$

	Days			
	7	14	28	73
Sludge tons/acre				
0	81.0%	78.9%	77.4%	55.9%
3	63.6	80.6	75.8	52.8
6	76.3	86.8	72.4	56.1
9	69.7	78.9	80.6	55.4
18	65.9	86.6	66.4	47.1
32	76.7	81.7	74.3	41.1
64	48.4	82.3	77.8	53.1
128	60.5	66.8	92.6	38.7
Average	67.8%	80.3%	77.2%	50.0%

PLANT GROWTH

High rates of sludge (64 and 128 tons/acre) prevented seed germination (Tables 56 and 57). No effect was apparent at lower sludge rates or without DnBP. Soybean height was unaffected by sludge or DnBP at 28 or 73 days. Soybean plant dry weight was not affected at 28 days but was significantly reduced at 73 days at higher sludge rates ($p \leq 0.10$). Soybean pod dry weight was not reduced by sludge rates at 73 days. Weed dry weight (*Panicum dichotomiflorum*, Fall panicum) at 73 days was extremely variable due to its absence in low sludge loading rate pots, but showed some increase with higher sludge rates.

PLANT UPTAKE

Total ^{14}C in soybean plants harvested at 28 days varied considerably (Figure 7) but was not significantly affected by sludge rate (Table 58). Extractable ^{14}C followed the same pattern. ^{14}C extraction efficiency averaged 52% for soybean plant (Table 59).

Total ^{14}C in soybean plants harvested at 73 days also varied considerably (Figure 8) and was not significantly affected by sludge rate. Soybean pods held lower ^{14}C uptake than plants with both total and extractable ^{14}C uptake unaffected by sludge rate.

Fall panicum (weed) uptake was lower than soybean pod. ^{14}C extraction efficiency averaged 37% for soybean plant, 65% for soybean pod and 55% for fall panicum (Table 60).

Uptake of ^{14}C DnBP was significantly affected by sludge rate and pot replicate in fall panicum (weeds) (Table 58). This is probably a reflection of the great variability in weed presence among the pots.

TABLE 56. NUMBER OF SOYBEAN PLANTS, HEIGHT (mm), DRY WEIGHT (gm) AND STANDARD DEVIATIONS OF PLANTS HARVESTED AT 28 DAYS

Sludge tons/acre	Number of Soybean Plants	Height (mm) Soybean Plants	Dry Weight (gm) Soybean Plants
Control	3	3.2 (0.4)	0.28 (0.21)
0	4	5.2 (1.1)	0.47 (0.08)
3	2	3.5 (2.1)	0.23 (0.05)
6	4	6.0 (1.4)	0.58 (0.33)
9	5	6.5 (2.1)	0.60 (0.18)
18	4	6.2 (2.5)	0.51 (0.03)
32	6	8 (.)	0.72 (0.03)
64	0	0	0
128	0	0	0
Statistics F	5.92**	1.13	1.96
Source of F Sludge rate	7.29***	1.36	2.39
DnBP Presence	1.62	1.12	1.24

** Statistically significant at $p \leq 0.05$
*** Statistically significant at $p \leq 0.01$

TABLE 57. NUMBER OF SOYBEAN PLANTS, HEIGHT (mm), DRY WEIGHT (gm) AND STANDARD DEVIATIONS OF PLANTS HARVESTED AT 73 DAYS

Sludge tons/ac.	No. of Soybean Plants	Height (mm) of Soybean Plants	Dry Weights (gm)		
			Soybean Plants	Soybean Pods	Panicum (sp.)
Control	3	23 (5)	3.48 (0.83)	0.74 (0.27)	0
0	4	23 (3)	3.76 (0.19)	0.92 (0.05)	0
3	5	22 (3)	4.02 (0.06)	0.97 (0.06)	0
6	2	24 (8)	3.22 (0.26)	0.66 (0.94)	0
9	2	18 (2)	2.15 (1.02)	0.74 (1.00)	3.16 (4.38)
18	1	13 (18)	1.36 (1.63)	0.27 (0.39)	0
32	2	16 (2)	1.35 (0.13)	0	6.21 (0.28)
64	0	0	0	0	5.28 (7.47)
128	0	0	0	0	10.73 (2.70)
Statistics					
F	3.24*	0.98	3.46*	0.29	2.66*
Source of F Sludge rate	4.03**	0.77	4.32**	0.34	3.42*
DnBP Presence	0.15	0.01	0.11	0.11	0

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* Statistically significant at $p \leq 0.10$
** Statistically significant at $p \leq 0.05$

FIGURE 7

Uptake (ppb) of Extractable, Oxidizable and Total 14C DnBP in Soybean Plants Grown in Sludge Amended Pots at 28 Days

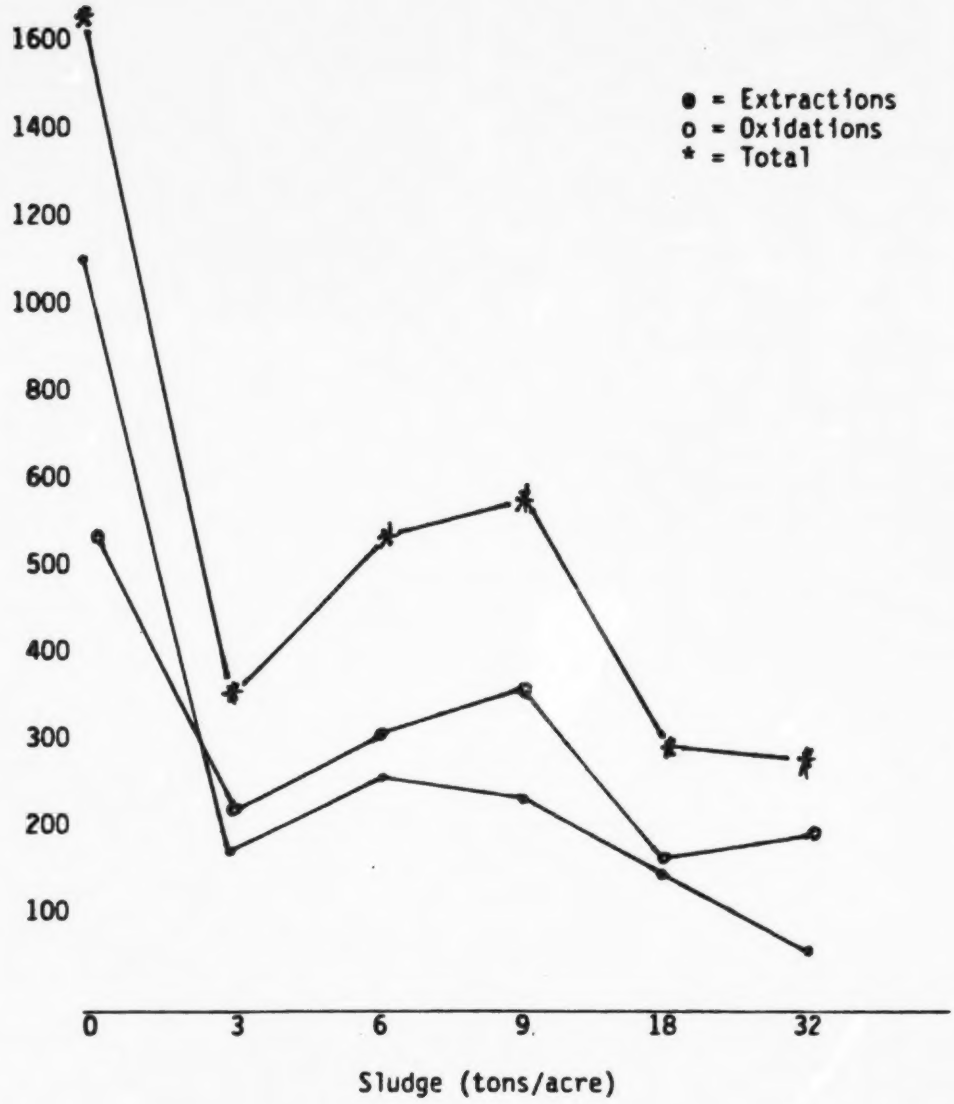

TABLE 58. SUMMARY OF STATISTICAL SIGNIFICANCE OF ^{14}C DnBP (extracted and total) IN PLANTS

	Green Soybean				Soybean Pods		Fall (weed) *Panicum* sp.
	28 day		73 day		73 day		73 day
	Extr.	Tot.	Extr.	Tot.	Extr.	Tot.	Extr.
F	0.43	0.22	0.82	0.39	0.32	0.74	6919.23***
Source of F							
Sludge rate	0.39	0.25	0.73	0.43	0.27	0.61	6697.25***
Rep	0.59	0.04	1.39	0.07	0.51	1.25	7585.19***

* Statistically significant at $p \leq 0.10$
** Statistically significant at $p \leq 0.05$
*** Statistically significant at $p \leq 0.01$

TABLE 59. PLANT ^{14}C EXTRACTION EFFICIENCY FROM 28 DAY PLANTS (%)

$$\frac{\text{dpm/gdw extractable}}{\text{dpm/gdw oxidizable}}$$

Sludge Rate tons/acre	Soybean Plant
0	67.2
3	42.8
6	44.9
9	36.2
18	48.6
32	19.4
Average	51.7

FIGURE 8

Uptake (ppb) of Total (oxidation and extraction) 14C DnBP in Plants Grown in Sludge Amended Pots at 73 Days

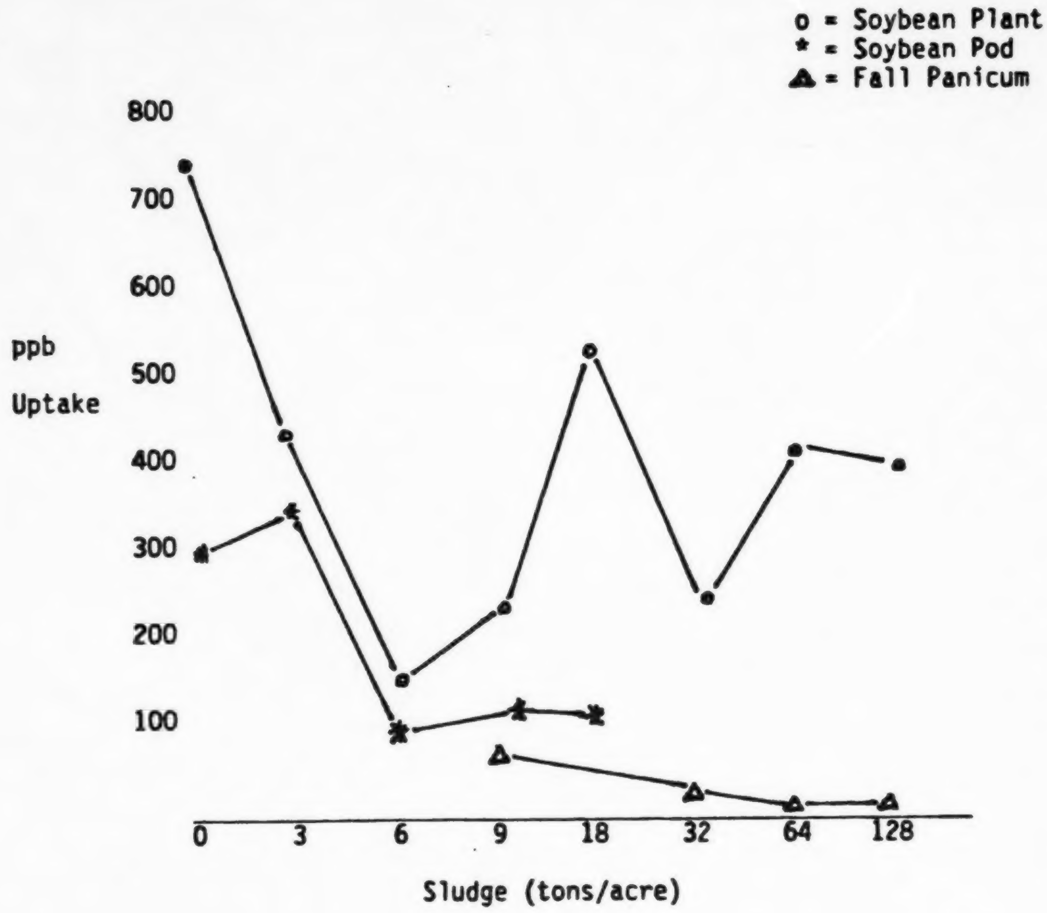

TABLE 60. PLANT ^{14}C EXTRACTION EFFICIENCIES FROM 73 DAY PLANTS (%)

$$\frac{\text{dpm/gdw extraction}}{\text{dpm/gdw oxidation and extraction}}$$

Sludge Rate tons/acre	Soybean Plants	Soybean Pods	*Panicum* sp.
0	38.0	66.6	-
3	47.4	61.0	-
6	33.0	57.1	-
9	34.5	82.4	53.2
18	44.4	56.8	-
32	28.4	-	63.9
64	23.5	-	50.0
128	-	-	46.2
Average	37.3	64.8	54.9

CONCLUSION

As time or sludge rate increased, the amount of soil ^{14}C DnBP (both extractable and total) decreased. Between 14 and 28 days a substantial decrease in soil ^{14}C DnBP occurred while at 73 days a smaller decrease was noted. ^{14}C DnBP levels were significantly reduced by time but only slightly affected ($p \leq 0.10$) by sludge rate. It does not appear that higher microbial populations associated with increased sludge greatly accelerated DnBP degradation. By 73 days, the amount of ^{14}C DnBP bound in the soil (50% unextractable) is approximately equal for all loading rates. This probably reflects a constant soil property for DnBP binding for this soil. It is likely that sludge rate will have little or no effect on soil degradation of ^{14}C DnBP beyond 73 days. Since the sludge rates (>18 tons/acre) showed no increase in total ^{14}C from 28 to 73 days, it is likely that total ^{14}C at lower sludge rates would approach 25% of the original amount in the future. Extractable ^{14}C would probably be near 10%. In previous work without sludge, extractable ^{14}C DnBP soil concentrations became constant by 30 days at about 9% of the original loading rate.

High rates of sludge (>64 tons/acre) inhibited soybean seed germination. No other effect was apparent on plant height or weight.

Uptake of total and extractable ^{14}C DnBP was not affected by sludge. Uptake was higher at 73 days than 28 days for plants grown in 3 and 18 tons/acre pots; otherwise, 73 day uptake was 42% of uptake at 28 days. Soybean pods had lower uptake than plants at 73 days. The lower uptake with more sludge reflects the pattern of soil ^{14}C DnBP.

In summary, sludge apparently has little effect on the degradation rate of ^{14}C DnBP added to soil. Eventually, soil ^{14}C DnBP concentration (both total and extractable) approach a level which is constant for the Norfolk soil. Soybean plant concentrations of ^{14}C DnBP reflected the soil concentration of ^{14}C DnBP. Uptake at 73 days was generally less than at 28 days.

SECTION 12

SUMMARY OF EXPERIMENTS

In this section, the goal is to assess whether there are conclusions regarding the behavior of organic compounds which evolve from the entire collection of experiments conducted. That is, important quantitative data were obtained in each separate chemical group studied that had been previously unavailable. However, the aggregate behavior of all chemicals leads to additional conclusions relative to municipal sludge land treatment.

As a selective display of the crop uptake experiments, data from all experiments are given in Table 61. One must remember certain facts regarding these data:

1. the most universal measurements made during these investigations were ^{14}C determination on extracts from soils or plant material;
2. measurements of ^{14}C contain both the original labeled parent compound and metabolites containing radiolabeled carbon;
3. some of the ^{14}C was not extractable.

With these constraints, Table 61 contains a measure of plant uptake, expressed as extractable ^{14}C, for the typical high loading rate (100X). This loading rate was usually 10,000-40,000 ppb. The soil concentration at the experiment end is a measure of soil level over the duration of the experiment, higher concentrations indicating more compound available for uptake. Water solubility is also given to provide a measure of availability in the water phase; lower solubility implies that, even if present, the chemical may be bound to the soil (lipophilic phase).

An initial conclusion is that probably all organic compounds have properties which permit uptake by vegetation. Such uptake occurs in natural soils and is limited by the degree of solubility and the magnitude of competing mechanisms (compound decomposition, volatilization, absorption, etc.) occurring in the soil. All compounds used in these experiments were found in some vegetation although for many plants no detectable concentrations were found. A similar conclusion can be reached concerning all inorganics (such as metals) and simply reflects the equilibrium distribution of all chemicals between soil and water with the subsequent dynamic processes by which plants take up water from the soil.

Comparison among the chemicals investigated of the ratio of plant concentration to soil level at the beginning of the experiment appears to argue against bioaccumulation. That is, this ratio was always less than 1.0 and most often less than 0.1. In the majority of cases, the ratio is

TABLE 61. SUMMARY OF SELECTED DATA FROM EXPERIMENTS IN THIS RESEARCH PROJECT

Compound	Water Solubility, mg/L	Soil Concentration at end of Experiment (100X), ppb (extractable ^{14}C)	Range of Plant Uptake, ppb as extractable ^{14}C (crop with maximum uptake)
di-*n*-octyl PAE	3	250	0-44 (fescue)
di-ethyl hexyl PAE	0.4	5,000	0-4,700 (corn)
di-*n*-butyl PAE	13	20-40	0-97 (corn)
di-*n*-butyl PAE	13	150	0-210 (soybean seed)
di ethyl PAE	900	170	0-220 (soybean plant)
di ethyl PAE	900	20	0-14 (soybean seed)
naphthalene	33	180-480	0-1,900 (corn)
anthracene	0.06	600-1,600	0-2,900 (wheat)
phenanthrene	1.2	380-620	0-30 (wheat)
benz(a)anthracene	0.01	4,000-6,000	0-190 (corn)
chlorobenzene	480	<1	0-23 (fescue)
p-dichlorobenzene	80	6	0-1,500 (fescue)

less than 0.01. This actual ratio is probably far lower since ^{14}C and not exclusively parent compound are being measured at the end of the experiment whereas the initial soil loading is clearly parent compound.

The factors affecting plant uptake are complex and involve competing soil behavior of each specific organic compound. If one qualitatively diagrammed the factors of relative availability for a chemical in a soil system and of relative loss by competing mechanisms, the crop uptake appears to have certain trends (Figure 9). For chemicals in which competing losses are high (i.e., volatile or microbially labile) the compound is not available for uptake. Likewise if a compound is present but not easily dissolved in water, the chemical will then be bound to the soil (the organic phase) and also not readily available. Conversely, compounds which are only slowly lost by competing pathways and are highly water soluble would be predicted to have generally higher rates of plant uptake. The compounds studied (Table 61) offer few, if any, exceptions to this qualitative behavior.

A final conclusion has only been partially evaluated by these experiments. Three compounds (chlorobenzene, p-dichlorobenzene, and di-n-butyl phthalate acid ester) were examined with and without the presence of large organic additions in the form of municipal sludge. For these chemicals the effect of the sludge was negligible or slight in regard to crop uptake or soil loss over the duration of the experiment. Should these data be verified on more chemicals, an important benefit would be derived. These are a large number of organic compounds which have been studied without sludge in plant-soil systems. The results of this research would indicate that as an initial estimation the literature data could be used to assess the behavior of a specific compound under municipal sludge land treatment.

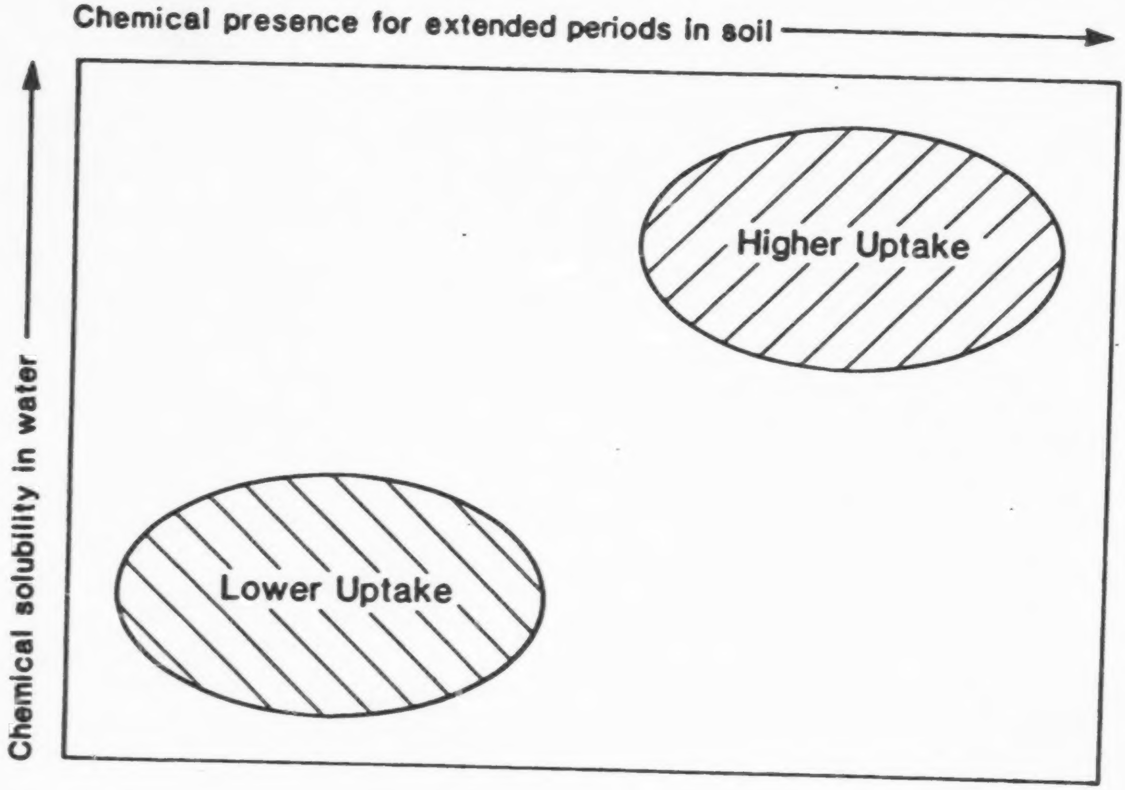
Chemical presence for extended periods in soil
Chemical solubility in water
Higher Uptake
Lower Uptake

Figure 9. Generalized relationship of organic chemical uptake by plants.